MAINTENANCE ENERGY REQUIREMENTS, POSTPARTUM REPRODUCTION, AND RUMINAL TEMPERATURE AT PARTURITION AND ESTRUS OF BEEF COWS

By

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

Figure Page

CHAPTER I

INTRODUCTION

Profitability of the beef cattle industry is highly impacted by reproduction. Cows must wean a calf every 12 months to be considered efficient. Maintenance energy requirement (MR) is a major factor influencing the profitability of beef production. Approximately 70% of the total annual energy required for beef cows is due to MR (Ferrell and Jenkins, 1984). Different strategies to optimize reproductive performance and to efficiently utilize feed energy in the cow-calf segment of beef production should be evaluated.

Major factors effecting reproduction are nutrition and suckling of calves. Nutrition during gestation and after calving impacts postpartum anestrus of beef cows. An extended postpartum anestrous interval reduces the efficiency of beef cattle production due to a decrease in the number and/or age of calves the next year. Body condition score at calving is a good indicator of future reproductive performance of beef cows. Body condition score estimates the energy reserves of a cow. Cows calving with moderate BCS have shorter postpartum intervals compared with cows calving in low BCS.

Postpartum nutrient intake and BCS at calving influences secretion of hormones that regulates reproduction. If body energy reserves are not adequate, pituitary hormones

are not secreted after parturition and this prolongs postpartum anestrus. The energy status of a cow can be indicated by concentrations of hormones in plasma such as insulin like growth factor I (IGF-I) and insulin. Plasma concentrations of IGF-I, insulin and glucose may participate in resumption of ovarian activity in beef cows (Wettemann et al., 2003)

Nutrient restriction decreases serum concentrations of IGF-I (Houseknecht et al., 1988; Richards et al., 1991) and increased nutrient intake increases IGF-I in heifers (Yelich et al., 1995), primiparous beef cows (Ciccioli et al., 2003) and gestating beef cows (Lents et al., 2005). Greater weight gain of primiparous lactating cows decreased the postpartum anestrous interval compared with cows with moderate weight gain (Ciccioli et al., 2003) or with cows that maintained BW (Rubio, 2005). Insulin like growth factor-I and insulin synergize with gonadotropins to stimulate ovarian steroidogenesis and follicular development (Spicer et al., 1993; Spicer and Echternkamp, 1995; Lucy, 2000), reduce postpartum anestrus (Roberts et al., 1997), and increase reproductive performance in beef cows (Ciccioli et al., 2003). Treatment with recombinant bovine somatotropin (bST) increases concentrations of IGF-I in plasma of lactating and nonlactating dairy cows (Bilby et al., 1999, 2004) and in serum of lactating beef cows (Armstrong et al., 1995; Flores et al., 2008). Treatment with bST increases milk production in dairy (Bauman and Veronon 1993) and beef cows (Armstrong el al. 1995). Milk production of beef cows is positively correlated with calf weaning weight (Neville, 1962). The use of bST and its impact on beef cow and calf performance is limited and requires further elucidation.

Another area that offers the opportunity to improve beef production is energy requirements for maintenance of beef cows. The cost associated with maintaining cows has a remarkable impact on profitability of beef production. About 50% of the energy required in the beef production systems goes to the cow herd (Ferrell and Jenkins, 1984). Differences in MR within and between cattle breeds and types have been recognized (Ferrell and Jenkins, 1984; DiCostanzo et al., 1990). In addition, heritability of MR is moderate (Hotovy et al., 1991). Therefore, identification and selection of beef cows with reduced MR, that wean a calf every year, should decrease inputs, increase production efficiency, and enhance the sustainability of the environment.

Traditional methods to determine maintenance energy requirements are expensive, time consuming, and are not practical for identification of more efficient cows. Identification of biomarkers for beef cows with low MR is essential to make progress in selecting for this trait. Metabolic hormones could be regulators of biological processes that are responsible for variation in MR. Nutrient intake influences concentrations of IGF-I, insulin, and T_4 in plasma of beef cows (Ciccioli et al., 2003; Lents et al., 2005). Rectal temperature has been associated with MR in beef steers (Derno et al., 2005) and mice (Kgwatalala et al., 2004). Available tools such as radioimmunoassay, rumen temperature boluses and proteomics analyses are techniques to investigate biomarkers for MR. Rumen boluses to record body temperature are practical and have minimal impact on animal behavior. Proteomics analyses describe protein expression at given times and conditions. Biomarkers may be discovered with the use of proteomics, without previous knowledge of the proteins and expression of the proteins (Lippolis and Reinhardt, 2008). Concentrations of hormones in plasma, body

temperature, and muscle protein expression in mature beef cows with different MR have not been determined.

Recent development of ruminal boluses to measure body temperature allows frequent collection of data in real time, with minimal impact on animal handling and behavior. Body temperature is related to physiological functions such as parturition and estrus in mammals. Body temperature decreases before parturition (Weber, 1910; Lammoglia et al., 1997; Aoki et al., 2005). A practical system to predict parturition may assist in decreasing calf loses. Dystocia is a problem in cattle (Bazer and First, 1983), and calf deaths can be partially prevented by obstetrical assistance (Bellows et al., 1987). Vaginal temperature increases during estrus in dairy (Bobowiec et al., 1990; Redden et al., 1993; Fisher et al., 2008) and beef cows (Kyle et al., 1998). Rate of genetic improvement of beef herds can be increased with the use of AI. Effectiveness of AI is highly influenced by a good estrous detection system. Systems which utilize body temperature changes to predict parturition or estrus are not commercially available because the technology required to record temperature has not been adequately developed (Rorie et al., 2002). An automated, frequent data collection system to determine temperature of cows must be practical, minimally invasive, and accurate to record body temperatures that can be associated with parturition and estrus. Evaluation of changes in ruminal temperatures during parturition and estrus requires further investigation.

Evaluation of different strategies to improve the profitability of the beef cattle industry is needed. Alternatives such as the use of bST in lactating nonpregnant beef cows, identification of beef cows with low MR, as well as the potential use of ruminal boluses to assess body temperature and predict parturition and estrus, should be

evaluated. Increased efficiency in the cow-calf segment of the beef industry is feasible. Weaning more kg of calves using similar resources or weaning the same kg of calves using less energy will improve efficiency of beef cattle production and enhance sustainability of the environment. Therefore the objectives for this dissertation were:

a) To evaluate the effects of postpartum weight gain and treatment with bST on calf growth and concentrations of IGF-I, glucose and insulin in plasma during early lactation.

b) To determine variation in MR of mature, nonlactating, beef cows during mid- to late-gestation.

c) To evaluate relationships among MR, cow performance, and postnatal calf growth.

d) To evaluate relationships among MR and plasma concentrations of IGF-I, thyroxine, glucose, insulin, and ruminal temperature.

e) To describe the proteome of *Longissimus dorsi* and evaluate protein abundance and potential biomarkers in mature beef cows with different MR.

f) To evaluate changes in ruminal temperature associated with parturition and estrus in spring-calving beef cows.

CHAPTER II

REVIEW OF LITERATURE

INTRODUCTION

Physiological mechanisms associated with reproductive processes of cows are complex and involve the hypothalamic-pituitary-ovarian axis. Among the main factors regulating reproduction, nutrition and suckling require the most attention. Nutrition and suckling of the calves affect hormones and metabolites that influence the hypothalamic-pituitary-ovarian axis and therefore reproduction. Nutritional status of the cow has an impact on the length of postpartum anestrus. Body condition score estimates the body energy reserves of a cow, and influences secretion of hormones that regulates reproduction. If energy reserves are not adequate, pituitary hormones are not adequately secreted after parturition which prolongs the postpartum anestrus. Growth hormone (GH) and IGF-I are metabolic hormones associated with the nutritional status of cows. The use of bovine somatotropin (bST) to increase milk production has been a common practice with dairy cows, and it has a diversity of effects on reproduction. However, little is know about how bST influences milk production, calf growth and reproduction in beef cows.

Maintenance energy requirements of the beef cow have a major impact on the cost of beef production. Approximately 70% of the energy required by beef cows

is required to meet MR. Selection of beef cows with lesser maintenance energy requirements is a feasible approach considering genetic variation and moderate heritability. Different methods are available to estimate MR, however those methods usually change natural conditions for the animals, are expensive and/or time consuming. Cows with low MR could be identified if biomarkers were available. Metabolic hormones, body temperature or certain proteins could be associated with MR and be potential biomarkers. Traditional and new tools can aid in identification of biomarkers for MR. Radioimmunoassay to determine concentrations of hormones in plasma, rumen boluses to measure body temperature, and proteomic analyses are available tools. This review will examine factors affecting reproduction in beef cows, with emphasis on estrus, GH and IGF-I, calf growth, MR of cattle, body temperature associated with parturition and estrus, and proteome analysis as a tool for animal science research.

FACTORS AFFECTING POSTPARTUM REPRODUCTION IN BEEF COWS

Nutrition

The first review of nutritional and endocrine control of reproduction was published in the Journal of Animal Science by Guilbert (1942). Later the impact of nutrition on reproduction was addressed by the classical work of Wiltbank et al. (1962). More recently the effects of nutrition on reproduction of beef cattle (Randel, 1990; Wettemann et al., 2003) and dairy cattle (Butler, 2000; Rhodes et al., 2003) have been reviewed. Reproduction is affected by nutrition primarily throughout a complex interaction of plasma hormones and metabolites that signal the brain resulting in an impact on the hypothalamic-pituitary-ovarian axis throughout GnRH secretion which controls the synthesis and secretion of luteinizing hormone (Short et al., 1990;

Wettemann et al., 2003; Hess et al., 2005). Hormones of greatest interest in reproduction are: growth hormone, IGF-I, insulin, leptin, NEFA and NPY (Wettemann et al., 2003; Hess et al., 2005). These metabolic signals are monitored by the brain and may have a direct or indirect impact on GnRH secretion, the pituitary gland and/or ovarian function (Randel, 1990; Wettemann et al., 2003). The exact mechanisms by which nutrition and these hormones and metabolites control reproduction are complex and require further examination.

Body condition at parturition

Body condition can be used to estimate body energy reserves of cows and, is determined using a scoring system which assigns values based on the amount of fat deposition on skeletal features (ribs, pin bones, spinus processes, tail head and brisket) as determined by palpation and sight of the animal. The most widely used scoring system for beef cows is described by Wagner et al. (1988), and uses a numerical scale to determine body condition score (BCS) from 1 (severely emaciated) to 9 (very obese). In beef cows, BCS predicted composition more accurately than body weight (Wagner et al., 1988). When back fat of beef cows with adequate condition was evaluated by ultrasound, it resulted in similar predictions as BCS (Renquist et al., 2006), however, this is not true for very thin cows. More over, BCS has a repeatability of 0.83 suggesting that the method is a precise system for evaluating beef cows (Vizcarra et al., 1995). Body condition score is a practical tool to make nutritional and management decisions for beef cattle production.

Body condition score at parturition is positively related with reproductive performance of beef cows (Richards et al., 1986; Selk et al., 1988; Osoro and Wright,

1992; DeRouen et al., 1994; Spitzer et al., 1995; Looper et al., 2003; Lake et al., 2005; Flores et al., 2007). Beef cows with a moderate BCS at parturition had a decreased interval from parturition to conception compared with cows with low BCS (Richards et al., 1986). Maintenance of a moderate BCS after parturition is also important. Beef cows that calved and maintained a moderate BCS, had greater pregnancy rates when compared with cows that lost BCS post partum (Rutter and Randel, 1984; Rakestraw et al., 1986).

Body condition score at parturition influences onset of luteal activity, the duration of the postpartum anestrous period, and pregnancy rate of beef cows. More primiparous beef cows with a BCS of 5 or 6 at calving had luteal activity by the end of the breeding season compared with cows with a BCS of 4 (Vizcarra et al., 1998). The percentage of primiparous (Spitzer et al., 1995) and multiparous beef cows (Flores et al., 2007) in estrus during the breeding season was greater for cows with greater BCS. Beef cows with BCS ≥ 5 had shorter postpartum anestrous interval (PPI) and shorter intervals from calving to pregnancy compared with cows with a BCS ≤ 4 (Richards et al., 1986). Osoro and Wright (1992) also found a decrease in time from parturition to conception in beef cows that calved with greater energy reserves. More recently, Looper et al. (2003) observed that Hereford and Angus x Hereford cows with BCS ≥ 4.5 had a decreased PPI compared with those with a BCS ≤ 4 (53 vs. 89 d). Similarly, Lents et al. (2008) found that beef cows calving with BCS ≥ 5 had shorter PPI compared with cows calving with BCS < 5. Pregnancy rates of primiparous and multiparous beef cows was greater for cows with greater BCS at parturition, and was 56 to 96% for cows with BCS of 4 to 7,

respectively (DeRouen et al., 1994; Spitzer et al., 1995; Lake et al., 2005; Lents et al., 2008).

Suckling

Postpartum reproductive performance of beef cows is effected by the frequency (Smith and Vincent, 1972; Laster et al., 1973; Bellows et al., 1974; Acosta et al., 1983) and intensity (Oxenreider, 1968; Wettemann et al., 1978; Randel, 1981; Mackey et al., 2000) of calf suckling.

Early weaning of calves decreased the PPI (Smith and Vincent, 1972) and increased the percentage of cows that conceived during the breeding season (Laster et al., 1973). In addition, BCS can influence the impact of early weaning on the PPI. Beef cows with a BCS < 5 had a greater interval to the onset of luteal activity, after early weaning when compared with cows with BCS ≥ 5 (Bishop et al., 1994).

The postpartum anestrous period was reduced in beef cows with weaned calves versus two calves (Oxenreider, 1968) and in cows that suckled one versus two calves, although BW was maintained for all cows (Wettemann et al., 1978). The postpartum anestrous period was also reduced in beef cows suckled once daily compared with ad libitum suckling (Randel, 1981). In addition temporary weaning (48 to 60 h) combined with estrous synchronization with a progestagen increased the number of cows detected in estrus and pregnant (Smith et al., 1979).

The presence of the calf and suckling increases the PPI by reducing LH pulses (Williams, 1990). Early weaning (Acosta et al., 1983), temporary weaning (Shively and Williams, 1989), restricted suckling (Mackey et al., 2000) and calf isolation (Stagg et al., 1998) increased LH pulse frequency resulting in improved reproductive performance of

the cows. The suckling stimulus suppresses GnRH release from the hypothalamus in Holstein cows (Zalesky et al., 1990), resulting in reduced LH release from the pituitary gland. Opioid peptides may participate in the inhibition of LH secretion (Myers et al., 1989) because when an opioid antagonist was administrated to suckled cows, LH secretion was increased (Whisnant et al., 1986b; Myers et al., 1989). The effect of opioid on LH secretion may become attenuated with days post partum (Whisnant et al., 1986a)

ESTRUS IN THE COW

Estrus or "heat" is the period of time at which the cow is sexually receptive, usually occurs every 21 d, but can vary from 17 to 24 d (Woody et al., 1967). During estrus a number of signs occur that constitute estrous behavior. The duration and expression of estrous behavior can be affected by different factors. Estrous duration ranges from 3 to 28 h in beef cows (White et al., 2002; Ciccioli et al., 2003; Lents et al., 2008; Floyd et al., 2009) and from 0.4 to 26.5 in dairy cows (Lopez et al., 2004). Study of estrous signs has been used to develop estrous detection aids and methods. Economical importance of estrous detection requires the design and evaluation of aids and methods that allow its improvement.

Behavior

Estrous behavior is the concurrence of different signs such as standing for mounting, mounting other cows, clear vaginal mucus and swollen vulva, which precede ovulation (French et al., 1989). Standing for mounting is the most reliable sign of estrus (Reimers et al., 1985; Senger, 1994; Sprecher et al., 1995). These signs have been used to visually detect estrus and to design different estrous detection methods.

Other signs accompany estrus such as changes in body temperature (Wrenn et al., 1958; Sampath and Iya, 1966) and milk temperature (Fordham et al., 1987; Fordham et al., 1988), increased physical activity (Kiddy, 1977; Lewis and Newman, 1984; Walton and King, 1986; Redden et al., 1993), decreased milk yield (Walton and King, 1986), and increased vaginal electrical conductivity (Bobowiec et al., 1990; Fisher et al., 2008). Increases of 0.3 to 0.9 ºC in body temperature have been observed during estrus in dairy heifers (Rajamahendran and Taylor, 1991), dairy cows (Wrenn et al., 1958; Zartman and Dealba, 1982; Rajamahendran et al., 1989; Bobowiec et al., 1990; Clapper et al., 1990; Redden et al., 1993; Piccione et al., 2003; Fisher et al., 2008) and beef cows (Kyle et al., 1998).

Environmental, genetic, physiological and social factors affect estrous behavior and its duration and expression in beef (Landaeta-Hernandez et al., 2002) and dairy cows (Gwazdauskas, 1985; Helmer and Britt, 1985; Rorie et al., 2002). Estrous behavior is influenced by season in direct or indirect ways (Gwazdauskas, 1985; White et al., 2002; Floyd et al., 2009). White et al. (2002) observed that duration of estrous in nonpregnant, nonlactating mature beef cows was greater during summer compared with winter (17.6 vs. 15.5 h) while the opposite (13.9 vs. 16.8 h) was reported by Floyd (2001). However, the number of mounts was greater during winter compared with summer (White et al., 2002; Floyd, 2001). Season had an effect on estrous behavior in dairy cows (Xu et al., 1998); winter and summer differed in estrous duration (9.7 vs. 7.3 h, respectively) and number of mounts (13.6 vs. 8.5, respectively). The concurrence of several females in estrus at the same time has an impact on estrous behavior. Duration, as well as number of mounts received during estrus, was greater for dairy heifers (Helmer and Britt, 1985),

dairy (Pennington et al., 1986) and beef cows (Flores et al., 2006; Floyd et al., 2009) when the number of females in estrus at the same time increased.

Breed influences estrous behavior. Angus heifers had greater estrous duration and number of mounts per estrus when compared with Brahman heifers (Rae et al., 1999). The interval from estrous synchronization to onset of estrus was greater for Brahman and Senepol compared with Angus cows (Landaeta-Hernandez et al., 2002).

Physiological status of the cow affects estrous behavior. Frequently the first estrus occurs after the first postpartum ovulation in beef cows (Short et al., 1990; Ciccioli et al., 2003). Most beef and dairy cows ovulate after the first estrus (King et al., 1976; Perry et al., 1991b; Ciccioli et al., 2003; Looper et al., 2003). Percentage of cows with estrous behavior increases with days post partum in dairy cattle (Pennington et al., 1986). Days after calving and/or lactation may effect the duration of estrus. Estrous duration was shorter (about 6 h) at the first estrus in lactating primiparous beef cows (Ciccioli et al., 2003), multiparous beef cows (Lents et al., 2008) and dairy cows (At-Taras and Spahr, 2001), while longer (13.9 to 17.2 h) estruses were observed in nonlactating beef cows after 5 mo post partum (White et al., 2002, Floyd, 2001). Milk production has an impact on estrous duration. Dairy cows with milk yield above the herd average had shorter (6.2 h) durations of estrus compared with cows with lower milk production (10.9 h). Also age of beef cows influenced the number of mounts but not the duration of estrus (Mathew et al., 1999).

Neither BCS nor protein supplementation influenced estrous duration or number of mounts received in postpartum Angus x Hereford cows (Lents et al., 2008). In

contrast, lesser BCS had a negative impact on the number of mounts per estrus in Brahman influenced cows (Flores et al., 2007).

Detection of Estrus

Estrous detection is one of the most important factors influencing profitability in dairy herds (Pecsok et al., 1994; Maatje et al., 1997). Missing the opportunity to AI a cow prolongs the calving interval and hence decreases milk production which has a negative impact on profitability of the production system. Similarly, inadequate estrous detection decreases profitability of beef production systems that use AI.

Conventionally, estrous detection occurs by visual observation of cows; however, this translates to high labor cost and tedious work (At-Taras and Spahr, 2001). Several aids have been designed to detect estrus or to facilitate the visual observation method. Some of the developed aids are: painting the tailhead or back of the cow with chalk, using a chin ball marker on a teaser bull or androgenized cow (Foote, 1975; Macmillan and Curnow, 1977; Sawyer et al., 1986; Senger, 1994), recording a video to achieve constant visual observation (King et al., 1976), measuring vaginal electrical resistance (Bobowiec et al., 1990; Fisher et al., 2008), using pedometers and activity meters (Kiddy, 1977; Pennington et al., 1986; Peralta et al., 2005), and more recently using pressure sensors and telemetry to determine riding behavior (Dohi et al., 1993; Senger, 1994; Stevenson et al., 1996; Rae et al., 1999; White et al., 2002; Floyd et al., 2009). Other signs have been studied to aid in estrous detection. Vaginal or rectal temperature, and thermal infrared scanning have been related to estrus with variable results (Zartman and Dealba, 1982; Zartman et al., 1983; Lewis and Newman, 1984; Rajamahendran et al., 1989; Mosher et al., 1990; Rajamahendran and Taylor, 1991; Redden et al., 1993). Most

studies have found an increase in body temperature during estrus. However, temperature measurements are often not practical, effective or accurate, and are not used as an indicator of estrus (Firk et al., 2002).

Efficiency and accuracy of different methods of estrous detection have been evaluated. Efficiency of estrous detection systems range from 49 to 98% for visual observation (Stevenson et al., 1996; Xu et al., 1998; Rorie et al., 2002; Peralta et al., 2005), from 44 to 96% for tailhead marking (Macmillan and Curnow, 1977; Sawyer et al., 1986), from 37 to 96% for the use of pedometers or activity meters (Lehrer et al., 1992; Senger, 1994; Peralta et al., 2005), and 48 to 100% for the use of pressure sensors and telemetry (Stevenson et al., 1996; Xu et al., 1998; Rorie et al., 2002; Peralta et al., 2005). Variability in the efficiency of estrous detection techniques may be due to the different conditions under which studies were conducted. Visual observation had an accuracy of 73 to 100%, pressure sensors and telemetry measures had an accuracy of 100% (Stevenson et al., 1996; Xu et al., 1998), and pedometers had an accuracy that ranged from 22 to 100% (Lehrer et al., 1992). The combination of different methods and aids frequently improves the efficiency of estrous detection to 80 or even 97% (Sawyer et al., 1986; Lehrer et al., 1992; Rorie et al., 2002; Peralta et al., 2005). Improving estrous detection methods, by increasing efficiency and accuracy, and reducing labor intensity and cost, is still a challenge that requires further investigation.

GROWTH HORMONE AND REPRODUCTION OF COWS

Growth hormone secretion and regulation

Growth hormone (GH) or somatotropin is a protein hormone containing 191 amino acids that is synthesized and secreted in a pulsatile manner by the somatotropes in

the anterior pituitary gland. Major controllers of GH are two hypothalamic hormones, growth hormone releasing hormone (GHRH) and somatostatin (Tuggle and Trenkle, 1996; Anderson et al., 2004). Somatotropes in the anterior pituitary, have receptors for GHRH and synthesize and release GH in response to GHRH binding (Tuggle and Trenkle, 1996). Growth hormone secretion is suppressed by binding of somatostatin to somatotropes (Tuggle and Trenkle, 1996) but synthesis is not inhibited (Anderson et al., 2004). More recently, it has been establish that ghrelin, acting via the GH secretagogue receptor, also stimulates GH secretion directly and indirectly by enhancing GHRH release from the hypothalamus (Anderson et al., 2004; Hashizume et al., 2005).

Other hormones from the hypothalamus and periphery also participate in the control and release of GH from the pituitary. Insulin-like growth factor I (IGF-I), leptin and thyroid releasing hormone (TRH) influence GH secretion (Anderson et al., 2004). Increased concentrations of IGF-I in plasma decreases GH secretion by negative feedback in a direct manner and indirectly by stimulating the release of somatostatin (Butler et al., 2003). Treatment of dairy (Gallo and Block, 1990) and beef cows (Armstrong et al., 1995) with bST increases concentrations of IGF-I in plasma.

Insulin-like growth factor I is a GH-dependent peptide with structure similar to insulin and IGF-II (Spicer and Echternkamp, 1995). Insulin-like growth factor I is primarily produced by the liver in response to GH binding (Jones and Clemmons, 1995; Keisler and Lucy, 1996).

Nutrition has an effect on plasma concentrations of GH and IGF-I in cattle. Cattle with a negative energy balance usually have increased GH and decreased IGF-I (Richards et al., 1991; Keisler and Lucy, 1996; Bossis et al., 1999). The inability of GH to

stimulate hepatic IGF-I secretion during nutritional deficiency is called “GH resistance”, uncoupling GH/IGF-I (Thissen et al., 1994; Donaghy and Baxter, 1996). Receptors for GH (GHR) in the liver are downregulated, therefore the normal stimulatory action of GH on the synthesis of IGF-I becomes uncoupled and IGF-I secretion by the liver is reduced despite high plasma concentrations of GH (Thissen et al., 1994). This situation is reversed during realimentation. Concentrations of hepatic GHR and plasma IGF-I are positively related to nutrient uptake (Donaghy and Baxter, 1996).

Concentrations of GH and IGF-I in plasma are effected by nutrition. Feed restricted beef cows (Kirby et al., 1993) and heifers (Houseknecht et al., 1988; Bossis et al., 1999) had increased concentrations of GH and decreased IGF-I in plasma . Beef heifers fed a nutritionally restricted diet to induce anovulation had reduced concentration of IGF-I in plasma before the onset of anovulation (Bossis et al., 1999). Concentrations of IGF-I in nutritionally induced anovulatory beef heifers increased during realimentation but were less than IGF-I concentrations in cyclic heifers at ovulation (Bossis et al., 1999); this increase in IGF-I was associated with increased diameter, growth rate and persistence of the dominant follicle (Buratini et al., 2000). In contrast, greater nutrient intake increased concentrations of IGF-I in plasma of beef heifers (Yelich et al., 1996; Armstrong et al., 2001), primiparous (Lalman et al., 2000; Ciccioli et al., 2003; Rubio, 2005) and multiparous beef cows (Richards et al., 1991).

Weight gain has an impact on plasma concentrations of IGF-I. Prepubertal beef heifers fed to gain 1.36 kg/d had greater concentrations of IGF-I in plasma compared with those fed to gain 0.23 kg/d before puberty (Yelich et al., 1995). Similarly primiparous prepartum beef heifers fed to gain 0.90 kg/d, had greater concentrations of

IGF-I in plasma compared with those fed to gain 0.45 kg/d after parturition (Ciccioli et al., 2003). Increases in BW and BCS have been associated with greater IGF-I in crossbred cows (Roberts et al., 2005).

A decrease in GH receptors (GHR) during negative energy balance may occur, probably due to an insulin-dependent downregulation (Thissen et al., 1994; Kobayashi et al., 1999; Butler et al., 2003). When insulin was infused into postpartum dairy cows, hepatic GHR-1A and IGF-I expression, and plasma IGF-I increased 3- to 6-fold, while insulin increased 8-fold compared with controls (Butler et al., 2003). There was a positive correlation between insulin and IGF-I in primiparous postpartum beef cows (Ciccioli et al., 2003; Rubio, 2005).

Energy reserves of postpartum beef cows are positively correlated to IGF-I; beef cows in greater BCS had greater concentrations of IGF-I in plasma (Bishop et al., 1994; Spicer et al., 2002; Ciccioli et al., 2003; Rubio, 2005; Flores et al., 2008). However, nutrient intake had a greater influence on IGF-I in plasma than BCS of gestating beef cows (Lents et al., 2005).

Concentrations of GH in plasma increase after parturition in dairy cows (Bell, 1995), while plasma concentrations of IGF-I (Taylor et al., 2004) and hepatic GHR-1A expression decrease (Kobayashi et al., 1999; Lucy et al., 2001; Radcliff et al., 2003). Concentrations of IGF-I in plasma gradually increases as lactation progresses, and hepatic GHR expression increases stimulating hepatic secretion of IGF-I (Sharma et al., 1994; Taylor et al., 2004). Concentrations of IGF-I in plasma of the dairy cows are greater during the dry period compared with lactation period (Sharma et al., 1994; Bilby et al., 1999; Thatcher et al., 2006).

Although expression of GHR-1A was reduced in periparturient dairy cows (Kobayashi et al., 1999; Radcliff et al., 2003), this does not occur in Angus cows (Jiang et al., 2005). Breed influences concentrations of IGF-I in serum of beef breeds during the postpartum period. Concentrations of IGF-I in serum increased between wk 2 and 7 post partum in Brahman cows and its Brahman crosses with Angus or Charolais, while no changes in concentrations of IGF-I in serum were observed in Angus, Charolais or Angus x Charolais cows during the same period (Spicer et al., 2002). Even though concentrations of IGF-I in plasma during the post partum period were influenced by breed, the number of days to the first medium or large follicle was similar for all breeds (Spicer et al., 2002).

Biological actions of growth hormone

The primary biological actions of GH in farm animals include growth, nutrient metabolism, reproductive function and lactation. These actions have been reviewed (Spicer and Echternkamp, 1995; Etherton and Bauman, 1998; Lucy, 2000; Renaville et al., 2002; Etherton, 2004) and can be direct, and/or indirect throughout IGF-I. Growth hormone stimulates bone and muscle growth, and fat mobilization directly and/or indirectly by IGF-I. Growth hormone promotes protein synthesis, decreases glucose uptake by muscle and fat, stimulates glucose output from the liver, and increases insulin secretion, creating an insulin resistance state (Mauras et al., 2000). Treatment of beef steers and heifers with somatotropin (bST) increases growth rate and feed efficiency (Moseley et al., 1992; Rausch et al., 2002).

Effects of GH on reproduction are also direct or through its mediator, IGF-I (Etherton, 2004). Perturbations of GH secretion in rodents and humans are associated

with impaired reproductive function (Hull and Harvey, 2001; Chandrashekar et al., 2004). Decreased concentrations of IGF-I in plasma of dairy cows compromise fertility (Pushpakumara et al., 2003).

Milk production can be increased by growth hormone treatment of mammals which affects partitioning of nutrients from adipose tissue to the mammary gland (Bauman and Vernon, 1993; Etherton and Bauman, 1998). Growth hormone has been used by the dairy industry to increase milk production. Advances in technology allowed the production of bovine bST by recombinant DNA technology (Etherton, 2004). Numerous studies have evaluated the effects of bST on cows. The commercial use of bST to increase milk yield in dairy cows began in 1994 in the USA, and milk yield is typically increased 3 to 5 kg/ (Burton et al., 1990; Bauman et al., 1999). In addition, the study of GH and IGF-I on biological actions of cattle have been facilitated.

Treatment of dairy cows (Gallo and Block, 1990; Bilby et al., 1999), dairy heifers (Radcliff et al., 2004), and beef cows (Armstrong et al., 1995; Flores et al., 2008) with bST increases plasma concentrations of GH and IGF-I. Concentrations of IGF-I in plasma of dairy and beef cows are increased after treatment with minimal amount (0.2, 12.5 or 25 mg/d) of bST (Gong et al., 1997; Gulay et al., 2004) or with the common commercial dose of 500 mg of slow release bST every 2 wk (Armstrong et al., 1995; Bilby et al., 2004; Flores et al., 2008).

When Holstein heifers were fed a high-gain diet and treated with bST, concentrations of GH in serum were decreased and concentrations of IGF-I in plasma were greater compared with heifers on a low-gain diet treated with bST (Radcliff et al., 2004). Beef cows with a greater BCS had greater concentrations of IGF-I in plasma after

treatment with bST compared with thinner cows (Flores et al., 2008). Since treatment with bST increased hepatic GHR-1A (Kobayashi et al., 1999) and IGF-I mRNA (Sharma et al., 1994) in cows, this indicates that the liver is responsible for the increase in plasma IGF-I (Sharma et al., 1994).

Growth hormone and reproduction of cows

Growth hormone is involved in sexual maturation, ovarian function and pregnancy (Chandrashekar et al., 2004). Actions of GH on reproductive functions of cattle are expected because receptors for GH have been found in reproductive tissues such as the corpus luteum, ovarian follicle, oviduct, uterus, and placenta (Lucy et al., 1993; Kolle et al., 1998; Lucy et al., 1998; Lebedeva et al., 2004). Actions of GH on reproduction may be direct or indirect by altering IGF-I secretion.

Luteal and ovarian function are influenced by GH and IGF-I. Cattle treated with bST had larger CL and thus greater concentrations of progesterone in plasma (Lucy et al., 1994). Ovarian function is stimulated by IGF-I and insulin, amplifying the action of gonadotropins on steroidogenesis and follicular growth and differentiation (Spicer et al., 1993; Spicer and Echternkamp, 1995; Spicer and Stewart, 1996; Lucy, 2000). Ovarian concentrations of IGF-I may originate in the liver or in the ovary, and follicular growth may be influenced by GH indirectly through IGF-I (Lucy, 2000).

Treatment of dairy (Gallo and Block, 1990; Bilby et al., 1999; Bilby et al., 2004; Bilby et al., 2006) and beef cows (Armstrong et al., 1995; Flores et al., 2008) with bST increases plasma concentrations of IGF-I. Positive effects of bST treatment on follicular development appear to be mediated through an increase in concentrations of IGF-I or

insulin in plasma, instead of a direct effect of bST on the ovary of heifers (Gong et al., 1997).

Treatment of cows with bST may stimulate follicular growth and maturation. Cows with GH receptor deficiency had less small follicles and failed to develop a first wave dominant follicle; this situation was accompanied by reduced concentrations of IGF-I in plasma (Chase et al., 1998). Treatment of Hereford x Fresian (Gong et al., 1991) and Nellore (Buratini et al., 2000) heifers with bST resulted in a greater number of small follicles compared with control heifers, and the increased number of follicles was associated with increased IGF-I in plasma. In contrast, the number of small follicles was greater in control compared with bST treated beef cows (Flores et al., 2008). Most studies to evaluate the effect of bST on reproductive performance of cows have been done with dairy cows. Treatment of lactating dairy cows with bST may be detrimental (Burton et al., 1990; Cole et al., 1992; Esteban et al., 1994; Luna-Dominguez et al., 2000; Bell et al., 2008), enhance (Moreira et al., 2000; Bilby et al., 2006; Starbuck et al., 2006), or have no effect (Hemken et al., 1991; Judge et al., 1999; Collier et al., 2001; Silvia et al., 2002; Jousan et al., 2007) on fertility. Days post partum at initiation of the treatment, level of milk production, parity, season, duration and dosage, may be some of the causes for the differences in response to bST treatment.

Early studies that evaluated the effect of bST treatments on reproduction found negative effects. An increase in the number of days open was observed in Holstein cows after treatment with different doses of bST (Burton et al., 1990; Cole et al., 1992; Esteban et al., 1994; Luna-Dominguez et al., 2000). A decrease in estrous detection rate may have been responsible for the increase in days open when primiparous Holstein cows

were treated with bST (Morbeck et al., 1991). The number of days after calving at which treatment is initiated may impact the number of inseminations required for pregnancy when multiparous Holstein cows are treated with bST. When bST treatment commenced during early lactation, the number of inseminations per conception were greater compared with when treatment was started in mid or late gestation (McGuffey et al., 1991). Energy balance of dairy cows may influence the response to bST treatment.

Other studies determined that treatment of dairy cows with bST may enhance fertility. Pregnancy rates were increased in lactating dairy cows treated once with bST at first insemination (Starbuck et al., 2006), two doses associated with a timed AI protocol (Bilby et al., 2006), or in cows treated at d 63 post partum and timed AI on d 105 post partum (Moreira et al., 2001).

Nonlactating dairy cows had greater basal concentrations of IGF-I in plasma compared with lactating cows (De la Sota et al., 1993; Bilby et al., 1999; Thatcher et al., 2006). Treatment with bST increased concentrations of IGF-I in plasma of lactating cows to concentrations similar to those in nonlactating cows (Bilby et al., 1999; Thatcher et al., 2006). Increased IGF-I after treatment with bST was greater in nonlactating cows compared with lactating cows (Thatcher et al., 2006), and treatment of nonlactating dairy cows with bST reduced pregnancy rate (Bilby et al., 2004; Thatcher et al., 2006). A deficient or an excess concentration of IGF-I in plasma may reduce reproductive performance (Thatcher et al., 2006).

Few studies have evaluated effects of bST treatment on beef cow reproduction. Treatment with bST, starting before (d 28 PP) or after (d 105 PP) the breeding season, did not influence luteal activity or pregnancy rates in beef cows, however treatment with bST

increased concentrations of IGF-I, glucose and insulin in plasma (Armstrong et al., 1995). Milk yield was increased in bST treated cows compared with controls, however nutrition management remained similar for both groups. Without increased nutrient intake bST treatment could have decreased the energy balance of treated cows and thus no effect on reproduction was observed (Armstrong et al., 1995). When using a single dose of bST at AI, Starbuk et al. (2006) found no effect on conception rates of beef cows with a good BCS (5 to 7 using the 1 to 9 scale). Flores et al. (2007) found a tendency for treatment with bST to reduce the postpartum interval in thin cows compared with moderate BCS beef cows. In addition first-service conception rate and pregnancy rate were increased for Brahman influenced beef cows treated with bST at the beginning of the breeding season compared with controls (Flores et al., 2007). Although the effect of bST on dairy cattle has been evaluated, the influence of bST on beef cow performance is limited. Evaluation of the effects of bST treatment and postpartum nutrition of postpartum beef cow on reproductive performance requires further investigation.

FACTORS INFLUENCING CALF GROWTH

Major factors influencing postnatal calf growth are sex of the calf, genetics and nutrition. Bull calves grow faster than steer calves (Marlowe and Gaines, 1958), and steer calves grow faster than heifer calves (Marlowe and Gaines, 1958; Neville, 1962). Breed of cows (Reynolds et al., 1978; Freetly and Cundiff, 1998), and cross breeding (Reynolds et al., 1978) influence calf growth rate and weaning weights, usually associated with the milk production potential of the dams.

Nutrition, particularly milk yield of the dam, is a major factor influencing calf growth. Greater calf weights at weaning are highly correlated with increased milk yield

(Neville, 1962; Totusek et al., 1973; Reynolds et al., 1978; Clutter and Nielsen, 1987; Perry et al., 1991a; Freking and Marshall, 1992; Marston et al., 1992). Cows with greater milk production weaned heavier calves, and the correlation between milk yield and calf average daily gain was 0.88 (Totusek et al., 1973). Variability in weaning weights due to milk yield of the dam was 66% (Neville, 1962) and 60% (Rutledge et al., 1971) in Hereford calves. Creep feeding of calves may decrease the correlation between milk production and weaning weight (Marshall et al., 1976).

The advantage of pre-weaning growth is reflected in post-weaning growth (Clutter and Nielsen, 1987). Calves from high milk production cows had greater weaning weight and maintained 63% of this advantage over those raised from less productive cows (Clutter and Nielsen, 1987). Breed of calf can affect cow milk production (Reynolds et al., 1978). Angus cows produced more milk when suckled by crossbred calves, and crossbred calves grew faster than purebred calves.

Differences in feed conversion efficiency exist among breeds and breed crosses. Crossbreed cows (F_1), with different potential for milk yield, had different efficiencies when expressed as the ratio of calf weight gain to energy consumed by the cow (Jenkins et al., 1991). Cows from Angus or Hereford dams crossed with Angus, Hereford, Red Poll or Maine Anjou sires were more efficient than F_1 cows produced by Chianina or Gelbvieh sires (Jenkins et al., 1991). Production efficiency of different breeds is influenced by feed availability. Cows with greater genetic potential for growth and/or milk production (Gelbvieh, Charolais, Braunvieh, Simmental, Pinzgauer, and Limousin) converted more feed to kg of weaned calves when there was excessive feed available,

whereas Red Poll, Angus and Pinzagauer were more efficient when less feed was accessible (Jenkins and Ferrell, 1994).

Use of bST increases milk yield in dairy cattle. Treatment of beef cows with bST increases calf growth (Armstrong et al., 1995), which is probably the result of increased milk yield. Treatment with bST of Angus, Charolais, and Simmental cows initiated on d 105 or 124 to 228 d post partum increased milk yield and calf weaning weight compared with cows treated from d 28 post partum or untreated cows (Armstrong et al. 1995). The mammary gland of early postpartum cows may not be very responsive to bST or young calves may not consume sufficient milk to stimulate secretory tissue. Additional research is needed to evaluate the effect of bST on beef cows and on calves.

MAINTENANCE ENERGY REQUIREMENTS OF CATTLE

Maintenance energy requirement (MR) has been defined as "the amount of feed energy intake that will result in no net loss or gain of energy from tissues of the animal body" (NRC, 1996). Another definition for MR (McDonald et al., 2002) is: "that which promotes energy equilibrium (zero energy balance)". Maintenance energy requirement can be estimated by different methods and is influenced by many factors. Reducing the MR per unit of body size is feasible given its genetic variation and moderate heritability (Carstens et al., 1989; DiCostanzo et al., 1990; Hotovy et al., 1991; Johnson et al., 2003).

Estimation of maintenance energy requirement

Maintenance energy requirement can be estimated by three main methods. These methods include feeding trials, calorimetric methods and comparative slaughter. Feeding trials consist of providing the animal a known amount of feed with a specific energy content. With this information, the quantity of energy required to maintain body weight

for an extended period of time can be determined. It is also possible to feed the animal and allow small gains or losses and then use a regression model including energy intake, live weight, and live weight gain, to determine the energy required for maintenance (McDonald et al., 2002). Feeding trials allow the animals to be managed under equal or similar conditions in a production system and large numbers of cattle can be evaluated.

Calorimetric methods allow estimation of maintenance energy requirements directly or indirectly. Measurement of heat production as a consequence of energy expended for maintenance is a direct measure. Quantifying respiratory exchange of the animal and oxygen consumption is an indirect estimate of heat production. This method is complicated and expensive as a calorimetric and/or respiratory chamber is required (McDonald et al., 2002). Management of animals in conditions that are very different from the natural environment and free-ranging conditions is another limitation of calorimetry (McDonald et al., 2002).

The comparative slaughter method measures initial and final body composition, energy intake, and energy retained, and maintenance requirements are calculated by difference. This method has the advantage of not interfering with normal conditions and behavior of the animal. However, the method requires an accurate estimation of body composition at the beginning and at the end of the trial, some animals must be sacrificed (NRC, 1996).

NRC (1996) adopted the California Net Energy System, proposed by Lofgreen and Garret in 1968 for beef cattle. This system is based on the comparative slaughter method, measures metabolizable energy (ME) intake and retained energy (RE), and heat production (HP) is calculated by difference. The ME intake at which RE equals zero

gives the estimate of ME for maintenance (NRC, 1996). The regression of the log of heat production (HE) on ME intake gives the intercept that estimates fasting heat production (FHP) which is equal to net energy requirements for maintenance (NE_m; NRC, 1996). The formula derivate from growing steers and heifers to calculate NE_m is:

$$NE_m = 0.077 \text{ Mcal/EBW}^{0.75}$$

were EBW is the average empty body weight in kilograms. However, this formula requires adjustments for type of animal if it is different than the one used to derive the formula (NRC, 1996). For beef cows, NRC (1996) adjusts the estimation of NE_m according to: breed, physiological state, activity, and ME intake vs. RE, BCS (Level 1 Model). The model also offers the opportunity to adjust for environmental conditions and animal insulation (NRC, 1996).

Measurement of heart rate has been used recently to determine energy expenditures by animals. Brosh et al. (2007) reviewed this method as an indicator of energy expenditure. Heart rate (HR) is highly correlated with ME intake and energy expenditure in ruminants (Brosh, 2007). Oxygen consumption and its relation with HR can be determined and oxygen consumption per heart beat, allows the calculation of energy expenditure (heat production) by the animal within their natural environment, such as grazing animals (Brosh, 2007). Further evaluations of this method in a variety of different conditions and species are required. In addition, this method is not available for producers due to the prohibitive price of HR monitors (Brosh, 2007).

Factors affecting maintenance energy requirement

Maintenance energy requirement is effected by different factors such as: breed and type, body condition score, body composition, environment, health, physical activity,

physiological status, previous nutrition, production level, size, sex, specie and visceral organ mass. These factors have been documented by Ferrell and Jenkins (1985), Crooker et al. (1991) and the National Research Council (NRC, 1996). Comparisons must consider differences in animals and conditions in which the studies were conducted. Factors affecting MR are discussed in the next sections.

Breed and type: Differences in MR of several breeds and types for different sexes were reported in 1911 by Armsby and Fries (cited by Ferrell and Jenkins, (1985b). Metabolizable energy requirement for maintenance (ME_m) ranged from 123 to 169 $Kcal \cdot BW^{-0.75} \cdot d^{-1}$ when evaluating MR of males and females from different breeds and breed crosses (Thompson et al., 1983; Ferrell and Jenkins, 1984, 1985b; Solis et al., 1988; Montano-Bermudez et al., 1990; Laurenz et al., 1991; Reid et al., 1991). Simmental bulls and heifers had greater ME_m than Herefords (Ferrell and Jenkins, 1985b). When evaluating bulls of different breeds and breeds crosses, differences in MR were found (Blaxter and Wainman, 1966; Garrett, 1971; Ferrell and Jenkins, 1985b). Ayrshire steers had 20% and 6% greater fasting heat production (FHP), respectively, when compared with Angus and Ayrshire x Beef Shorthorn steers (Blaxter and Wainman, 1966). Similarly Holstein steers had 12% greater MR compared with Hereford steers (Garrett, 1971). Hereford bulls had 14% less FHP when compared with Simmental bulls (Ferrell and Jenkins, 1985b). Differences in MR of cows are usually associated with milk production, and cows with greater milk production have greater MR. Angus x Hereford cows had lesser ME_m compared with Angus x Holstein cows (128 vs 140 $Kcal \cdot BW^{-0.75} \cdot d^{-1}$; Thompson et al., 1983). Ferrell and Jenkins (1984) observed ME_m ($Kcal \cdot BW^{-0.75} \cdot d^{-1}$) of 130, 129, 145 and 160 for Angus x Hereford, Charolais x Hereford or Angus, Jersey x

Hereford or Angus, and Simmental x Hereford or Angus mature cows, respectively. Cows with greater milk production potential had greater ME_m, with no effect of cow size on ME_m. Comparing beef (Angus, Brahman and Hereford) with dairy breeds (Holstein and Jersey), ME_m ranged from 92 to 140 $Kcal \cdot BW^{-0.75} \cdot d^{-1}$, respectively (Solis et al., 1988). Similarly, Montano-Bermudez et al. (1990) found different ME_m, for low, medium and high milk production potential cows, and ME_m was associated with milk potential. Laurenz et al. (1991) observed greater MEm for Simmental compared with Angus nonpregnant, nonlactating cows (134 vs. 124 $Kcal \cdot BW^{-0.75} \cdot d^{-1}$, respectively). Most of these findings have been estimated in different conditions using different methodologies therefore direct comparisons among studies are not possible. Nevertheless, NRC (1996) reported that several generalizations can be made; dairy breeds of *Bos taurus* require 20% more energy for maintenance than beef breeds and crossbreeds are intermediate.

Body composition: Differences in body composition, or BCS as an indicator of the body composition affect MR. Muscle tissue has greater impact on MR than fat tissue in swine (Tess et al., 1984; Noblet et al., 1999), cattle (Webster, 1977; DiCostanzo et al., 1990; Baker et al., 1991) and other species (Webster, 1977). Nevertheless fat tissue provides insulation under cold environments and reduces the energy required for maintenance in pigs and cattle during winter (Thompson et al., 1983; Tess et al., 1984; Wagner et al., 1988).

Lean mass was a better predictor of heat production than fat mass in pigs from different breeds, however visceral organ mass had a greater influence on heat production than lean mass (Tess et al., 1984; Noblet et al., 1999). Maintenance energy requirements

were greater for nonlactating nonpregnant Angus cows with a greater proportion of lean body mass compared with cows with less lean body mass (DiCostanzo et al., 1990). This difference has been attributed to the greater energy requirements to maintain lean tissue and for protein turnover (Webster, 1980; Tess et al., 1984; Lobley, 2002).

Evaluations made during winter found that fat cows have lesser ME_m than thin cows, and greater insulation for fat cows may be responsible for the difference (Thompson et al., 1983). Maintenance energy requirements (ME_m) were greater (6.1%) for thin compared with fat Angus x Hereford cows (131.5 vs. 123.5 $Kcal \cdot BW^{-0.75} \cdot d^{-1}$, respectively). In contrast, fat Angus x Holstein cows had greater (2.7%) MR compared with thin cows (142.4 vs. 138.6 Kcal $Kcal \cdot BW^{-0.75} \cdot d^{-1}$, respectively). Dairy type animals have greater deposition of fat internally than externally and hence less insulation during winter (Thompson et al., 1983).

Mature, nonpregnant, nonlactating Hereford cows in moderate BCS (5) had greater ME_m (4.4% or 8.9%) compared with thin (BCS = 3) or fat (BCS = 7) cows, and thin and fat cows had similar requirements during winter (Wagner et al., 1988). Lesser MR in thin cows may be associated with a decrease in visceral organ mass (Wagner et al., 1988; Freetly et al., 2006) and/or decreased thyroxine in plasma (Rasby et al., 1991). Fat cows had a lesser MR than moderate cows and this may be attributed to greater insulation provided by fat along with greater heat production from tissues of the gastrointestinal tract, considering the increased feed intake (FI) to sustain BW (Wagner et al., 1988).

Visceral organ mass: There is a positive correlation between visceral organ mass and MR. Data from different studies with cattle (Ferrell and Jenkins, 1984; DiCostanzo et al., 1990; Reynolds et al., 1991), sheep (Burrin et al., 1990) and pigs (Tess

et al., 1984; Noblet et al., 1999) support this concept. Cows of different types and sizes with greater visceral organ mass had greater ME_m, with no effect of cow size (Ferrell and Jenkins, 1984). Greater visceral organ mass was associated with greater milk production potential (Ferrell and Jenkins, 1984).

When visceral organ mass was increased in lambs (Burrin et al., 1990) and beef heifers (Reynolds et al., 1991), oxygen consumption and hence heat production increased. Lambs with ad libitum feed had increased visceral organ size and oxygen consumption compared with those fed to maintain BW (Burrin et al., 1990). Reynolds et al. (1991) induced changes in visceral organ mass by altering fiber content of diets, and observed that a greater visceral organ mass resulted in greater heat production. DiConstanzo et al. (1990) found that MEm was positively correlated with liver weights and the relative portion of heart and liver to empty body. Portal-drained viscera had increased oxygen consumption when pregnant ewes had greater feed intake and oxygen consumption from the liver accounted for an estimated 40% of total heat production (Freetly and Ferrell, 1997)

Visceral organs, specially the liver and gastrointestinal tract, are responsible for a high proportion of the energy expenditure of the whole body (Ferrell and Jenkins, 1985a; McBride and Kelly, 1990). Synthesis of protein in visceral organs is greater than in muscle (Lobley et al., 1980; Crooker et al., 1991) and contributes to the energy expenditure in these organs (Lobley et al., 1980; Lobley, 2002). Liver and the gastrointestinal tract constitute around 8 to 14% of the total body protein, and synthesize 25 to 45% of the total body protein; whereas skeletal muscle constitutes approximately 50% of the total body protein and only synthesize 15 to 20% of total body protein

(Lobley, 2002). Visceral organs of pigs contribute three times more to ME_m when compared with lean mass (Noblet et al., 1999).

Environment: Changes in the environment due to location and/or season have an impact on MR of animals which is usually associated with feed availability and ambient temperature changes (Ferrell and Jenkins, 1985a; Laurenz et al., 1991; Jenkins and Ferrell, 1994b; Calegare et al., 2007). Angus and Simmental cows had greater ME_m in the summer compared with the winter (122.6 vs 91.4 and 145.9 vs 109.3 $Kcal \cdot BW^{-0.75} \cdot d^{-1}$, respectively). Differences in ME_m can be attributed to changes in body composition along with energy required for body temperature homeostasis (Laurenz et al., 1991).

Beef cows with greater genetic potential for productivity may express that potential in better nutritional environments (Ferrell and Jenkins, 1985a; Jenkins and Ferrell, 1994, 2007). Biological production efficiency (weight of weaned $calf \cdot cow^{-1} \cdot$ kg DMI of cow^{-1}) among nine different breeds of cattle varied with feed intake (Jenkins and Ferrell, 1994). The response of the different breeds to DMI was curvilinear and differed among the breeds. Red Poll cattle were more efficient at DMI < 4,000 kg/yr, while breeds with greater genetic potential for growth and/or milk production (Gelbvieh, Charolais, Braunvieh, Simmental, Pinzgauer, and Limousin) were more efficient at DMI > 6,000 kg/yr, and Angus and Hereford were intermediate (Jenkins and Ferrell, 1994). Nellore cows had lesser energy requirements compared with Nellore x Angus and Nellore x Simmental, and Nellore cows were less efficient (ADG of $calf \cdot$kg DMI of cow-$calf^{-1}$) compared with Nellore x Angus and Nellore x Simmental in an advantageous nutritional environment (Calegare et al., 2007). Crossbreeding improved preweaning biological efficiency when cows were in an environment without nutritional constraints (Calegare et

al., 2007). Genetic potential and environmental conditions, particularly feed availability, must be matched to get the best of each situation, and use of high genetic potential cows in a nutritionally limited environment will have a negative impact on production efficiency, primarily by reduced reproduction (Jenkins and Ferrell, 1994a; Calegare et al., 2007)

Health: Maintenance energy requirements increase when a disease or infectious process occurs. The immune response requires energy and consequently increases MR (NRC, 1996). Activation of the immune system is supported by energy from the feed and/or the body tissues (NRC, 1996).

Physiological state: Lactating Hereford cows had 31 % and 41% greater MR than nonlactating cows (Neville and McCullough, 1969; Neville, 1974). Evaluation of MR of beef cows from different crosses (Angus, Hereford, Red Poll and Milking Shorthorn) during two consecutive gestations, determined that ME_m was about 18% less during the nonlactating, gestating state compared with the lactating nonpregnant state (Montano-Bermudez et al., 1990).

Feed allowance: Feed allowance, whether below or above maintenance requirements, has an impact on heat production. Previous plane of nutrition and compensatory gain affect MR (NRC, 1996). Response to feed restriction varies according to severity and duration of the restriction, type of restricted nutrient (s), animal genotype, and other factors (NRC, 1996).

Heat production decreases with feed restriction of beef steers (Murphy and Loerch, 1994), beef heifers (Yambayamba et al., 1996; Freetly et al., 2003) nonpregnant nonlactating beef cows (Freetly et al., 2006), and pregnant cows (Freetly et al., 2008).

The decrease in heat production may be associated with decrease of secretion of thyroid hormones and IGF-I, increased GH, and reduced size of visceral organs during restriction (Hornick et al., 2000). Metabolic changes remain for 30 to 125 d after realimentation (Yambayamba et al., 1996; Hornick et al., 2000).

Daily heat production is dynamic and positively associated with FI in nonlactating, nonpregnant (Freetly and Nienaber, 1998; Freetly et al., 2006) and pregnant (Freetly et al., 2008) beef cows. Metabolic rates of cows fluctuate as a consequence of changes in nutrient availability. Mature nonlactating, nonpregnant cows with restricted FI will adapt, and heat production will decrease until the cow is adapted to less FI and returns to maintenance. This adaptation occurred around 112 d after feed restriction to 35% below maintenance (Freetly et al., 2006). Similarly, during realimentation heat production increases regardless of the previous feed restriction and the FI (Freetly and Nienaber, 1998; Freetly et al., 2006). Pregnant beef cows had a similar response to adaptation to feed restriction and realimentation even though the restriction was not as severe as in previous studies to avoid a negative impact on fetal development (Freetly et al., 2008). Restriction of FI during the second trimester of gestation, and allowing cows to recover BW during the third trimester, did not effect cow BW at parturition or calf production (Freetly et al., 2008). Estimation of MR of mature cows will be influenced by the method used (Freetly et al., 2006).

Sex: Maintenance energy requirement of bulls is 15% greater compared with MR of heifers and steers of the same genotype (NRC, 1996). Hereford x Friesian cross bulls had greater predicted basal metabolism (20%) when compared with steers (Webster et al., 1977). Ferrell and Jenkins (1985) estimated that ME_m was 11 % greater for bulls

compared with Hereford and Simmental females. Net energy for maintenance for heifers, steers, and bulls of Nellore and crosses with different breeds, were not influenced by sex (Calegare et al., 2007; Chizzotti et al., 2008). Bulls in the study were individually fed and their physical activity was restricted, which could have influenced the lack of a sex effect on MR (Calegare et al., 2007; Chizzotti et al., 2008).

Species: Growing *Bos indicus* require cattle 10% less energy than *B. Taurus*, and crossbreeds are intermediate (NRC, 1996). Nellore cows had lesser ME_m compared with crosses with *B. taurus* (Calegare et al., 2007). Maintenance energy requirement was lesser for Brahman x Hereford compared with Red Poll cows (Reid et al., 1991).

Other factors: Cows with an aggressive temperament may expend more energy maintaining the nervous state (Crooker et al., 1991). Physical activity increases heat production and grazing animals have greater MR compared with penned cattle (NRC, 1996). The estimated cost of grazing activity, associated with locomotion during grazing, was 2.92 cal/(kg of $BW^{.75}$.m) for beef cows (Brosh et al., 2006).

Variation and heritability of maintenance energy requirement

Maintenance energy requirement varied by 5 to 35% among and within different breeds (DiConstanzo et al., 1990; Johnson et al., 2003; Derno et al., 2005). The greatest difference in ME_m was 26.6% for Angus cows (DiCostanzo et al., 1990) and 22.8% for Hereford steers (Derno et al., 2005). In monozygous twin beef cattle (Angus x Hereford and Barzona x Hereford), heritability for ME_m was 0.71 and 0.49 at 9 and 20 mo of age (Carstens et al., 1987; Carstens et al., 1989). Similarly, Hotovy et al. (1991) used identical twins (Angus x Hereford and Barzona x Hereford) to find a moderate heritability (0.52) for ME_m. The heritability for maintenance (0.22) was less for Hereford

bull calves (Bishop, 1992). The heritability for heat production ($Kcal \cdot BW^{-0.75} \cdot d^{-1}$) in mice that were selected 15 generations for heat production, was 0.31 (Nielsen et al., 1997a; Nielsen et al., 1997b). Therefore, reducing MR per unit of body size in cattle seems to be feasible, considering the variation and moderate heritability for this trait.

BODY TEMPERATURE AND PHYSIOLOGICAL FUNCTIONS

Body temperature is an indicator of different biological functions and health of domestic animals. Changes in body temperature have been associated with parturition since 1910, when Weber reported a decrease in rectal temperature of cows before parturition. Wrenn (1958) found that rectal temperature of cows decreased before parturition and increased at estrus. More recently the use of radiotelemetry allowed frequent determination of body temperature, and a decrease in body temperature prior to parturition (Lammoglia et al., 1997) as well as an increase during estrus were confirmed (Clapper et al., 1990). Nevertheless, practical methods to determine body temperature to predict parturition and estrus are not available. Body temperature has also been associated with maintenance energy requirement. Mice with high maintenance energy requirements had greater rectal temperature compared with low maintenance energy requirement mice (Kgwatalala et al., 2004). Similarly, rectal temperature was associated with maintenance energy requirement of beef steers (Derno et al., 2005).

Development of new technology allows determination of body temperature with the use of rumen boluses. Remote collection of ruminal temperature brings the opportunity to collect real time data, with minimal or no change in the behavior or natural conditions of ruminants. The absence of stress allows collection of physiological temperatures. In addition, rumen boluses are easy to recover at harvest. Ruminal

temperature has been associated with health, ambient temperature and water consumption (Dye, 2005; Ipema, 2008), thus making rumen boluses a potential alternative to monitor not only health status but other physiological functions in ruminants with minimal impact on behavior.

Measurement of body temperature

The common method to determine core body temperature of animals is by use of a rectal thermometer. Different methods, in addition to thermometer, have been used to evaluate changes in body temperature associated with different physiological functions. Some of the non-traditional methods to measure body temperature are injected/implanted transponders, tympanic devices, mounted rectal probes, vaginal and ear radiotransmiters, thermal infrared scanning and rumen boluses.

Electronic temperature monitors have been placed under the *Obliquus internus abdominis* muscle, to evaluate changes in body temperature associated with parturition in beef cows (Lammoglia et al., 1997). A decrease in body temperature occurred before parturition (Lamoglia et al., 1997). Similarly, implanting a transmitter in the abdominal muscles determined an increase in temperature during estrus of dairy cows (Zartman and Dealba, 1982).

Temperature sensing probes inserted in the tympanic membrane of the ears of animals have been used to evaluate the changes in body temperature after chilled water consumption by dairy cows (Stermer et al., 1986), different types of calf housing (Macaulay et al., 1995) and exercise (Mader et al., 2005). Mean tympanic temperature increased from 0.3 to 0.8 °C after moving feedlot cattle 600 m (Mader et al., 2005). Likewise, ear skin temperature has been measured using radiotransmiters anchored to the

ventral surface of the ear, however, a relationship between ear temperature and estrus in dairy cows was not detected (Redden et al., 1993).

Rectal probes allow continuous monitoring of rectal temperature for a short period of time and have been used to evaluate body temperatures of beef steers under heat stress conditions (Brown-Brandl et al., 2003). Vaginal probes were used to determine that body temperature decreases prior to parturition in beef cows (Aoki et al., 2005).

Infrared scanning was not efficient or accurate for detection of estrus in dairy cows (Hurnik et al., 1985). However, infrared scanning was able to predict health status of beef steers (Schaefer et al., 2007).

Recently, ruminal temperature boluses have been used to monitor body temperature in cattle. Ruminal temperatures are transmitted by radio waves to a computer and data are easily available for analyses. Ruminal temperatures can be determined at frequencies of seconds or hours (Dye, 2005). Ruminal temperature determined with ruminal boluses is positively correlated with rectal temperature ($R^2 = 0.8$), and ruminal temperature was 0.13 ºC less than rectal temperature in beef steers (Dye, 2005).

Ruminal temperature boluses were used to evaluate ruminal temperature change in beef steers exposed to bovine viral diarrhea virus (BVDV), and challenged with bovine respiratory disease (BRD). Ruminal temperature increased from 0.8 to 1.3 ºC after treatment of steers, indicating that ruminal boluses have the potential for detecting temperature changes associated with health events (Dye, 2005).

Changes in ruminal temperature associated with water consumption have also been appraised using boluses in sheep (Brod et al., 1982), beef steer (Dye, 2005) and

dairy cows (Bewley et al., 2008; Ipema et al., 2008). Ruminal temperatures decreased with water consumption and the magnitude of the decrease was dependent on volume and temperature of consumed water (Brod et al., 1982; Dye, 2005; Bewley et al., 2008). Consumption of water with temperatures ranging from 0 to 33 ºC caused a decrease in ruminal temperature that ranged from 2 to 7 ºC in sheep (Brod et al., 1982). Similarly, consumption of 0.83 to 14.2 L of water with temperatures from 16.2 to 23 ºC by steers, resulted in a 0.5 to 7.5 ºC decrease in ruminal temperature, with an average decrease of 1.9 ºC (Dye, 2005). Consumption of water at body temperature, or cold water, caused a decrease in ruminal temperature of 0.4 or 9.2 ºC, respectively, in dairy cows (Bewley et al., 2008). Ruminal temperature returned to pre-water consumption temperatures in 15 min when consumed water was at body temperature, but more than 3 h was required when cold water was consumed (Bewley et al., 2008). Diurnal variation in ruminal temperature has been observed in beef steers (Dye, 2005) and dairy cows (Ipema et al., 2008). Ruminal temperatures are usually less during the day and greater during the night (Dye, 2005; Ipema et al., 2008).

Retention of the bolus in the reticulorumen after oral administration has been a concern. Ghirardi et al. (2006) found that weight and volume of the bolus are crucial features that influences the rate of regurgitation or passage of boluses; heavier boluses were retained better than lighter boluses.

Different methods to estimate body temperature have been used to evaluate biological processes of animals. The availability of new technologies allows a better estimation of body temperature associated with different physiological functions and health.

Body temperature at parturition

Body temperature at parturition was evaluated in 1910 and Weber found a decrease in temperature of cows 28 h before parturition. Body temperature before parturition can decrease from 0.4 to 1.0 ºC in dairy (Vollmann and Vollmann, 1942; Wrenn et al., 1958; Ewbank, 1963; Aoki et al., 2005) and beef cows (Dufty, 1971; Lammoglia et al., 1997). A decrease in body temperature before parturition of cows was detected when a rectal thermometer was used to measure temperature once or twice a day (Wrenn et al., 1958; Ewbank, 1963). Use of more sophisticated methods, that record body temperatures frequently (every 1-3 min), have confirmed that body temperature decreases before parturition (Lammoglia et al., 1997; Aoki et al., 2005). Body temperature decreased 48 h before parturition in beef cows that were implanted with an electronic temperature monitor under the *Obliquus internus abdominis* muscle (Lammoglia et al., 1997). Vaginal temperature decreased ≥ 0.3 ºC from 60 to 72 h before parturition in dairy cows, and prediction of parturition based on a decrease in temperature was 100% effective (Aoki et al., 2005).

Changes in body temperature of gestating beef cows were associated with time of the day, sex of the calf, and parturition (Lammoglia et al., 1997), however, sex of the calf did not influenced temperature of dairy cows (Aoki et al., 2005). Greater body temperatures were observed with heavier calves at birth (Lammoglia et al., 1997; Aoki et al., 2005). Heavier calves may have heavier placentas which generate more heat and impact body temperature (Lammoglia et al., 1997). Additionally, the decrease in body temperature during 48 h before parturition was independent of average ambient temperature (Lammoglia et al., 1997).

Body temperature changes during the periparturient period of cows may be associated with metabolic adaptation or endocrine and behavioral changes. Periparturient hormonal changes have been reviewed (Bazer and First, 1983) but the hormones and mechanisms participating in the decrease of body temperature before parturition have not been elucidated.

Wrenn et al. (1958), proposed that changes in body temperature of cows were similar to those in women in which progesterone has a thermogenic effect. Vaginal temperatures were greater during pregnancy and during the luteal phase of the estrous cycle compared with temperatures 1 to 2 d before parturition and at estrus in dairy cows (Wtenn et al., 1958). Treatment of ovariectomized cows with progestagens increased body temperature compared with controls (Wrenn et al., 1958). Body temperature was correlated with concentrations of progesterone in plasma, and progesterone predicted 91% of parturitions in dairy cows (Birgel et al., 1994).

Changes in concentrations of progesterone, estradiol-17β, thyroxine (T_4), triiodothyronine (T_3), and $PGF_{2\alpha}$ in plasma prior to parturition may influence the decrease in body temperature (Lammoglia et al., 1997). However, only 30% of the variation in temperature at parturition was accounted for changes in hormone concentrations (Lammoglia et al., 1997). Hormones with the greater impacts on body temperature were T_3 and $PGF_{2\alpha}$. Concentrations of thyroid hormones during the periparturient period are not established in cattle.

Behavioral changes of cows before parturition may have an impact on plasma concentrations of thyroid hormones and on the heat generated due to ruminal fermentation. Prior to parturition in cattle, feed and water consumption are reduced

(Grummer et al., 2004; Lukas et al., 2008). Feed restriction decreases plasma concentrations of T_4 and realimentation increases T_4 in plasma of nonlactating beef cows (Richards et al., 1995). Greater BW gain increases plasma concentrations of T_4 in suckled postpartum beef cows (Ciccioli et al., 2003) and steers (Hersom et al., 2004). There is diurnal variation in plasma concentrations of T_4, with an increase at or shortly after feeding (Bitman et al., 1984). Changes in T_4 prior to parturition in cows have not been established, but concentrations of in T_4 serum of Holstein heifers at 90, 180 and 260 d of pregnancy were similar (Hernandez et al., 1972). Perhaps the decrease in FI prior to parturition is adequate to decrease secretion of thyroid hormones and reduce the calorigenic effect.

Decreased feed and water consumption prior to parturition may impact ruminal fermentation, amount of heat generated and body temperature. Evaluation of changes in ruminal temperature associated with feed consumption are limited and the effect of different diets on ruminal temperature requires further study (Dye, 2005).

Although several studies identified a decrease in body temperature prior parturition in cows, a practical method to predict parturition using body temperature has not been developed.

Body temperature at estrus

Vaginal and rectal temperature increased in dairy cows during estrus, when temperature was measured with a thermometer once a day (Vollmann and Vollmann, 1942; Wrenn et al., 1958; Sampath and Iya, 1966). Vollman and Vollman (1942) found an increase in only 55 to 60% of the cows while Wrenn et al. (1958) observed an increase in body temperature in 90% of cows.

No changes in body temperature associated with estrus (Lewis and Newman, 1984; Hurnik et al., 1985), an increase in body temperature during estrus and also an increase in temperature of non-estrous cows (Walton and King, 1986), or an increase in body temperature associated with estrus (Zartman and Dealba, 1982; Zartman et al., 1983; Rajamahendran et al., 1989; Clapper et al., 1990; Mosher et al., 1990; Redden et al., 1993; Kyle et al., 1998; Piccione et al., 2003; Fisher et al., 2008) have been reported. Experimental design, methodology used to measure body temperature, frequency of data collection, physiological status of the females, and environmental conditions may be responsible for variation in results.

Body temperature increased 0.6 to 0.8 ºC during estrus in Holstein cows implanted with a probe in the abdominals muscles that recorded temperatures once each day (Zartman and Dealba, 1982). All cows that were inseminated on the day ± 1 d of the increase in body temperature became pregnant (Zartman and Dealba, 1982).

Mean vaginal temperature increased 0.5 ºC greater than the average of the previous 5 d for spontaneous estrus but not for synchronized estrus, when vaginal temperatures was assessed once each day (Zartman et al., 1983). Vaginal temperature increased at estrus when estrous cycles were synchronized with $PGF_{2\alpha}$ and temperatures were recorded every 4 h (Rajamahendran et al., 1989). The frequency of data collection may be in part responsible for the differences in the detection of changes in body temperature at estrus.

Vaginal and rectal temperatures were highly correlated with standing estrus (r = 0.82 and 0.81, respectively), and onset of standing estrus was highly correlated (r = 0.96) with peak LH (Rajamahendran et al., 1989). An increase in vaginal temperature,

recorded by radiotelemetry, was associated with the LH surge in lactating dairy cows (Clapper et al., 1990), cycling dairy heifers (Mosher et al., 1990) and nonlactating dairy cows (Fisher et al., 2008). The interval from LH surge to the increase in vaginal temperature and the duration of the increase differed among the studies. When vaginal temperature was determined every 4 h, an increase ≥ 0.3 ºC for 8.1 h, at 17 h after LH surge occurred in lactating dairy cows (Clapper et al., 1990). When vaginal temperature was determined every 15 min, an increase ≥ 0.3 ºC for 11.4 h, at 5.2 h after LH surge, was observed in cycling dairy heifers (Mosher et al., 1990). Vaginal temperature, assessed every second, increased within 4 h of the LH surge in 76% of cows, or around 6 h from LH surge in 14% of nonlactating dairy cows (Fisher et al., 2008).

Parity had no effect on the increase in vaginal temperature associated with estrus in dairy cows when temperature was measured every 4 min by radiotelemetry. The increase was ≥ 0.3 ºC compared with the previous 4 d, and the duration of the increase was 6.8 h (Redden et al., 1993).

Kyle et al. (1998) evaluated vaginal temperature of multiparous suckled beef cows measured every 4 min by radiotelemetry. An increase in vaginal temperature ≥ 0.4 ºC occurred for 3 or more hours, compared with the previous 3 d, and duration of the increase was 6.5 h (Kyle et al., 1998).

The fact that the increase in vaginal temperatures occurs for only 4 to 11.4 h partially explains previous findings in which body temperature was not associated with estrus when temperature was measured once a day (Lewis and Newman, 1984). The short duration of the increase in body temperature at estrus may be the reason that some studied identified non-estrus cows as estrus (Walton and King, 1986). An increase in

body temperature of some cows may be missed when body temperature is determined once or even twice a day.

Throughout the estrous cycle, body temperature decreased during proestrus and increased during estrus in dairy and beef cows (Wrenn et al., 1958; Bobowiec et al., 1990; Kyle et al., 1998). Wrenn et al. (1958) and Bobowiec et al. (1990) found greater vaginal temperatures during the luteal phase, followed by a decrease two day before the increase in temperature at estrus in cyclic dairy cows. Similarly Kyle et al. (1998) observed greater vaginal temperatures during mid-cycle and at estrus in beef cows.

Similar to body temperature at parturition, many factors may influence body temperature at estrus in cattle. We speculate that water consumption, physical activity and endocrine secretions may be some of the factors that are responsible for variation in body temperature at estrus.

Behavior of cows change with estrus; physical activity increases (Kiddy, 1977; Pennington et al., 1986), water consumption is reduced (Meyer et al., 2004; Lukas et al., 2008), and FI is altered. Physical activity stimulated an increase in body temperature in steers that walked 600 m (Mader et al., 2005), and cows that had homosexual physical activity associated with estrus had increased body temperature (Walton and King, 1986). The effect of estrus on FI is inconsistent. Estrus of dairy cows did not influence FI (De Silva et al., 1981), decrease the time expended feeding (Diskin and Sreenan, 2000), or increase FI (Lukas et al., 2008). Changes in FI may influence fermentation and heat generated in the rumen.

Hormonal changes before, during and after estrus may have an impact on body temperature of cows (Wrenn et al., 1958). Increased estradiol during estrus may have an

impact on body temperature. Treatment of ovariectomized dairy cows with estradiol-17β increased uterine blood flow (Roman-Ponce et al., 1978). Uterine blood flow increased during estrus in the sheep (Roman-Ponce et al., 1983), cows (Bollwein et al., 2000) and mares (Bollwein et al., 2002). The increase in UBF during 48 h before to 24 h after estrus was first observed (visual detection every 12 h) to be negatively associated with concentrations of progesterone in plasma and positively associated with the estrogen (estradiol and estrone) to progesterone ratio in sheep (Roman-Ponce et al., 1983). Treatment of ovariectomized dairy cows with estradiol-17β under heat stress conditions increased UBF by 412 % when cows had shade available or by 287 % when shade was not available for cows (Roman-Ponce et al., 1978). This difference indicates that cows with no shade available redirected blood flow towards the body surface for heat dissipation.

There may be a positive association between increased body temperature and the LH peak (Rajamahendran et al., 1989; Clapper et al., 1990; Mosher et al., 1990; Fisher et al., 2008). Consequently an increase in estrogen, and the corresponding increases in LH, could be partially responsible for the increase in body temperature of heifers and cows.

The effect of ambient temperature on body temperature fluctuations during estrus is not clear. Most studies have been conducted when cows are exposed to ambient temperatures within or close to the thermo neutral zone or ambient temperature was not reported. Ambient temperature seems to have a minimal effect on body temperature of cows, unless animals are exposed to very hot or cold temperatures (Zartman and Dealba, 1982). When ambient temperature decreased, an increment in body temperatures was observed in cows, this possibly relates to adjustment in plasma concentrations of

thyroxine several days after the decrease (Zartman and Dealba, 1982). In contrast, a greater variation in vaginal temperature, due to ambient temperature, was observed compared with changes during the estrous cycle of dairy cows (Lewis and Newman, 1984).

The increase in body temperature during estrus has been detected with numerous methods. Nevertheless, a practical application of this indicator is not available. A non-invasive system to assess body temperatures with low or no impact on animal behavior, and one that allows frequent readings, is required for a practical application of this knowledge. Further investigations of the application of rumen boluses to determine body temperature and to use this information to predict parturition and estrous detection of cows are needed.

PROTEOME ANALYSIS AS A TOOL IN ANIMAL SCIENCE RESEARCH

Proteome is defined as the conjunction of proteins in a tissue at any given time (Sheffield and Gavinski, 2003), and study of the proteome is named proteomics (Wasinger et al., 1995). Proteomics determine the protein expression at a given time and conditions, and can analyze thousands of proteins in a single experiment with the goal of discovering differences in protein expression, protein interactions or modifications as a result of experimental treatment or conditions (Lippolis and Reinhardt, 2008). Therefore, possible biomarkers may be discovered with the use of proteomics and previous knowledge of the proteome is not required (Lippolis and Reinhardt, 2008).

Proteomics analysis includes several steps. After extraction, proteins are separated to allow comparisons of their abundance and identification. Two-dimensional SDS PAGE (2D-PAGE) is the most common type of gel electrophoresis and is a major

advance in proteomics (Sheffield and Gavinski, 2003). It allows separation of proteins according to two physical characteristics, isoelectric point and molecular weight (Hoorn et al., 2006). Proteins are initially separated by their isoelectric points in an isoelectric focusing gel (first dimension), and then proteins are transferred to a SDS-PAGE gel to be separated (second dimension) by molecular weight (Hoorn et al., 2006; Lippolis and Reinhardt, 2008). Spots in the gel are visualized by different methods, and converted to digital images using scanning devices for analyses. An image analysis program can detect and quantify the volume of spots to be matched and compare spots and expression in different gels (Karp and Lilley, 2005).

Running parallel 2D-PAGE is a common method to quantify and compare protein expression, but requires technical replication to overcome gel to gel variation which leads to problematic detection and quantification of differences in protein expression (Karp and Lilley, 2005). This inconvenience has recently been resolved by using protein dyes called CyDye fluors that label proteins before 2D-PAGE (Hoorn et al., 2006). This new method is named difference gel electrophoresis or DIGE, and was validated in 2001 using two different dyes for two samples in the same gel (Tonge et al., 2001). Later the method was improved by incorporating a third dye for an internal standard. Two samples from different treatments and a standard (pooled aliquots from all samples) can be included in the same gel; the standard will be present in every gel with the purpose of normalizing and standardizing data, to minimize technical variation and improve accuracy of quantification (Karp and Lilley, 2005; Hoorn et al., 2006). Technical replicates are not used but biological replicates are sufficient for quantification and comparisons of protein expression (Karp and Lilley, 2005).

Proteins can be rapidly identified by mass spectrometry. There are several types of mass spectrometers, and the matrix assisted laser desorption ionization (MALDI) is the method of choice (Sheffield and Gavinski, 2003). The mass spectrometer is an instrument that measures molecular weight of an ion (Hoorn et al., 2006). The basic process is an initial digestion using trypsin, ionization of peptides be detected in the mass spectrometer to be detected, isolated, fragmented and sequenced by the mass spectrometer (Lippolis and Reinhardt, 2008). Protein identification software is used to match peptides with trypsinized theoretical proteins from known protein sequences in a database (Hoorn et al., 2006).

The use of proteomics in animal sciences is in "its infancy" (Lippolis and Reinhardt, 2008). This methodology has been applied in the study of animal health, dairy foods, muscle biology, reproduction (Lippolis and Reinhardt, 2008), and energy metabolism (Kuhla et al., 2007).

Proteomics methods identified glucocorticoids induced proteins that could increase the susceptibility of calves to respiratory disease (Mitchell et al., 2007). There is a differential expression of liver proteins involved in different functions when evaluating ketosis in cows; different proteins involved in the pathogenesis of bovines were identified (Xu and Wang, 2008). The protein to modify or selection of bacteria for optimum dairy food production is of major importance in the field of dairy products. Protein synthesis of different bacteria has been assessed (Rechinger et al., 2000). To better understand the biochemical processes during post mortem storage of meat, the bovine muscle proteome has been studied. Proteomics have been used to map proteins in specific muscles (Bouley

et al., 2004) or to identified changes in the abundance of metabolic enzymes, or defense and stress proteins (Jia et al., 2006a; Jia et al., 2007).

Reproduction in animal production systems has major impact on profitability; proteomics analyses allow understanding of signaling processing, and identifies possible biomarkers for fertility. To better understand the signaling mechanisms between the trophoblast and the endometrium, embryo-induced alterations in the proteome of the bovine endometrium have been evaluated during the preattachment period (Berendt et al., 2005). Evaluation of seminal fluid (Kelly et al., 2006; Moura et al., 2006) and spermatozoal proteins (Peddinti et al., 2008) has also been addressed with the proteomics approach. Potential molecular markers associated with fertility in bulls were identified (Peddinti et al., 2008). Cows with different feed allowances had proteins that were differentially expressed in the hypothalamus. This technique identified possible molecular factors involved in energy homeostasis in cows (Kuhla et al., 2007).

Proteomics allows generation of data that will be difficult to obtain by other methods. Its application in animal sciences will help in the understanding of changes in the cells in response to changes in environments (Lippolis and Reinhardt, 2008). Protein expression in the muscle of beef cows with different maintenance energy requirements has not been investigated.

SUMMARY

Nutrition has a major influence on reproductive performance of beef cows. Nutrient intake during the early postpartum period influences the length of postpartum anestrus. Understanding endocrine changes associated with nutrient intake and the impact on resumption of luteal activity after parturition is of major importance. Changes in

metabolic hormones and metabolites such as IGF-I, insulin and glucose, during the reestablishment of ovarian activity have been studied. Treatment with bST increases IGF-I and milk production in dairy cows. Endocrine changes that lead to increased milk production after treatment with bST could also have an impact on reproduction in beef cows. The effect of nutrient intake during the postpartum period and the use of bST on reproduction and calf performance in beef cows requires evaluation. Efficiency of beef production could also be increased by reducing the energy required for maintenance of the cow herd. The energy consumed for maintenance of cows represents more than the 50% of the variable cost in beef production. It is feasible to decrease MR since it has moderate heritability. Identification of more efficient cows by measuring MR using traditional methods is expensive and/or time consuming. Evaluation of metabolic hormones, body temperature, and muscle protein expression of cows with different MR are some of the possibilities to find biomarkers for MR. Evaluation of possible biomarkers for MR requires the reduction of the influence of other factors on MR. In addition, evaluation of the performance of cows that differ in MR, and also calf performance, is necessary. Improvements in the cow-calf segment of the beef industry could involve decreasing the postpartum anestrous period, increasing preweaning calf growth, decreasing MR of the cow herd while maintaining cow and calf performance, and improve efficiency and accuracy of estrous detection. Therefore the objectives for this dissertation were:

a) To evaluate the effects of postpartum weight gain and treatment with bST on calf growth and concentrations of IGF-I, glucose and insulin in plasma during early lactation.

b) To determine variation in MR of mature, nonlactating, beef cows during mid- to late-gestation.

c) To evaluate relationships among MR, cow performance, and postnatal calf growth.

d) To evaluate relationships among MR and plasma concentrations of IGF-I, thyroxine, glucose, insulin, and ruminal temperature.

e) To describe the proteome of *Longissimus dorsi* and evaluate protein abundance and potential biomarkers in mature beef cows with different MR.

f) To evaluate changes in ruminal temperature associated with parturition and estrus in spring-calving beef cows.

CHAPTER III

EFFECT OF BODY WEIGHT GAIN AND BOVINE SOMATOTROPIN TREATMENT ON CALF GROWTH AND CONCENTRATIONS OF IGF-I AND INSULIN IN POSTPARTUM BEEF COWS

ABSTRACT: Angus x Hereford cows (2 and 3 yr of age, n = 37) were used to evaluate the effects of postpartum weight gain and treatment with bovine somatotropin (bST) on calf growth and concentrations of IGF-I, glucose and insulin in plasma during early lactation. Cows (456 ± 52 kg, BCS = 4.6 ± 0.4) were stratified based on calving date and BCS at calving, and randomly assigned to a 2 x 2 factorial: weight gain (WG) ≤ 0.4 kg/d (M) or > 0.40 kg/d (H), and cows were injected with bST (250 mg; POSILAC®, Elanco, IN) or saline (C) on d 31 and 45 post partum. Concentrations of IGF-I, insulin and glucose were quantified in plasma collected twice weekly, from d 24 until d 59 after calving. Data were analyzed using the MIXED procedure of SAS. Before bST treatment, H had greater (P = 0.02) concentrations of IGF-I in plasma compared with M cows (42.3 vs. 30.6 ± 3.7 ng/mL). After bST treatment, there was a WG x bST x day effect (P < 0.01) on plasma IGF-I. Concentrations of IGF-I in plasma were greater (P < 0.01) in HbST compared with MbST, MC or HC cows on 3, 7 and 10 d, after the bST treatments. Concentrations of glucose in plasma before bST treatment, were greater (P =

0.06) for H compared with M cows (70.0 ± 1.5 vs 65.8 ± 1.5 mg/dL, respectively). After bST treatment, concentrations of glucose in plasma were greater (P = 0.005) in HbST (72.4 ± 1.5 mg/dL) compared with HC, MC, and MbST cows (68.2 ± 1.6, 65.7 ± 1.5 and 64.2 ± 1.6 mg/dL, respectively). Concentrations of insulin were greater (P = 0.04) in H (1.57 ± 0.34 ng/mL) compared with the M cows (1.34 ± 0.34 ng/mL) before treatment with bST. After bST treatment, concentrations of insulin in plasma were greater (P < 0.01) in H (1.74 ± 0.35 ng/mL) compared with M cows (1.25 ± 0.35 ng/mL) on d 34 and during d 45 to d 59 post partum. Weight gain and treatment with bST did not influence (P = 0.99) the percentage of cows with luteal activity by 59 d post partum or the subsequent calving interval (P > 0.3). Average daily gain of calves to 140 d of age was greater ($P \leq 0.06$) for bST and H treated compared with C and M cows, respectively, and ADG to 220 d of age was greater (P = 0.01) for H compared with M cows. Weight gain of young lactating beef cows influenced plasma concentrations of IGF-I and glucose after treatment with bST. Weight gain and bST treatment of cows influenced ADG of calves. Growth rate of calves can be enhanced by increased postpartum weight gain and treatment of young beef cows with bST.

Key Words: beef cow, body weight gain, somatotropin.

INTRODUCTION

Reproductive performance of young beef cows is influenced by BCS at calving (Spitzer et al., 1995), postpartum nutrient intake (Vizcarra et al., 1998; Ciccioli et al., 2003, Rubio, 2005), and suckling of calves (Stagg et al., 1998). Nutrient restriction decreases serum concentrations of IGF-I (Houseknecht et al., 1988; Richards et al., 1991), and increased nutrient intake increases IGF-I in heifers (Yelich et al., 1995),

primiparous beef cows (Lalman et al., 2000; Ciccioli et al., 2003) and gestating beef cows (Lents et al., 2005). Greater weight gain of primiparous lactating cows decreased the postpartum anestrous interval compared with cows with moderate weight gain (Spitzer et al., 1995; Ciccioli et al., 2003). Treatment with recombinant bovine somatotropin (bST) increases concentrations of IGF-I in plasma of lactating and nonlactating dairy cows (Bilby et al., 1999, 2004) and increases IGF-I in serum of lactating beef cows (Armstrong et al., 1995; Flores et al., 2008). Insulin-like growth factor-I and insulin synergize with gonadotropins to stimulate ovarian steroidogenesis and follicular development in vivo (Lucy, 2000) and in vitro (Spicer et al., 1993), and IGF-I in plasma is positively associated with incidence of estrous cycles (Roberts et al., 1997) and reproductive performance of postpartum beef cows (Ciccioli et al., 2003). Insulin, IGF-I, and glucose may be associated in resumption of ovarian activity in beef cows (Wettemann et al., 2003)

Treatment with bST increases milk production in dairy (Bauman and Veronon, 1993) and beef cows (Armstrong el al., 1995). Milk production of beef cows is positively correlated with weaning weight of calves (Neville, 1962; Rutledge et al., 1971). The increase in milk yield is a consequence of increased nutrient availability for the mammary gland, accompanied with increased cell numbers and blood flow to the mammary gland (Burton et al., 1990; Bauman and Vernon, 1993). Treatment of cows with bST induces lipogenesis or lipolysis (Bauman and Vernon, 1993; Etherton, 2004), reduces glucose oxidation, and increases gluconeogenesis in the liver, and decreases insulin-induced glucose uptake by the muscle, thus increasing available nutrients and

glucose in plasma for mammary gland milk synthesis (Bauman and Vernon, 1993; Butler and Le Roith, 2001).

We hypothesized that greater body weight gain of young postpartum beef cows and treatment with bST would have synergistic effects to increase concentrations of IGF-I, glucose, and insulin in plasma, improve reproductive performance, and increase milk production and ADG of calves. The objective of this study was to evaluate the effects of postpartum weight gain and treatment with bST on calf growth and concentrations of IGF-I, glucose and insulin in plasma during early lactation.

MATERIALS AND METHODS

Animals, diets and treatments

The Oklahoma State University Animal Care and Use Committee approved all the experimental procedures used in this study. Spring-calving Angus x Hereford beef cows (456 ± 52 kg, BCS = 4.6 ± 0.4, n = 37, 2 and 3 yr of age) were stratified at calving by BCS and calving date, and randomly assigned to one of two weight gain groups during d 2 to d 59 after calving. Cows had *ad libitum* access to prairie hay (6% CP) and were fed to achieve a moderate (M, ≤ 0.4 kg/d, n = 18) or high weight gain (H, > 0.4 kg/d, n = 19). Moderate weight gain cows received 1.8 kg/d of a 38% CP supplement and H cows had high energy ration (1.61 Mcal NE_m/kg DM, 0.9 Mcal NE_g/kg DM, and 11.1% CP) composed (DM basis) of: rolled corn (39.7%), ground alfalfa pellets (35.5%), cottonseed hulls (22%), sugar cane molasses (2.5%) and salt (0.3%) ad libitum. Cows on H consumed an average of 24 kg of feed/cow/d. Cows grazed native range pasture (*Andropogon scoparius, Andropogon gerardii*) and were exposed to fertile bulls after d 60 post partum. Calves were castrated at birth by banding (Lents et al., 2006).

Cows in each weight gain group were randomly assigned to receive bST (250 mg, s.c., POSILAC®, Elanco, IN) or saline (s.c.) on 31 ± 4 d (d 0) and 45 ± 4 d after parturition. Injections were administered in the depression on the side of the tailhead; one was on the right side and the other on the left side. Calves remained with dams continuously.

Body weight and BCS

Body weight of cows, after denied water and feed for 16 h, and BCS (1 = emaciated to 9 = obese; Wagner et al., 1988) were recorded on d 24 ± 4 and 59 ± 4 post partum. Calves weights were recorded at birth and on d 60 ± 4 of age. Body weight of cows and calves, and BCS of cows were recorded on d 100, 137, 171 and 218 ± 10 after calving. Weaning weights of calves were recorded on d 221 ± 10 of age after denied water and feed for 16 h.

Blood samples, hormones and assays

Blood samples were collected twice a week for 5 wk, commencing 1 wk before the first bST treatment. Samples were taken by puncture of caudal veins into vacutainer tubes containing EDTA, stored on ice, centrifuged at 2500 x g for 20 min at 4°C within 3 h after collection, and plasma was recovered and stored at -20°C until analyzed. On the days of bST or saline treatments, blood was collected prior to treatment.

Plasma concentrations of IGF-I and glucose were quantified in all samples, and insulin was determined in weekly samples and at 3 d after administration of bST or saline. Plasma concentrations of progesterone were quantified in samples taken twice weekly from 35 d postpartum until luteal activity was confirmed. The criterion for luteal

activity was 2 or more blood samples collected at a 3 or 4 d interval with concentrations of progesterone greater than 1 ng/mL (Wettemann et al., 1972).

Concentrations of IGF-I in plasma were determined after acid ethanol extraction (16 h at 4 ºC) by RIA (Echternkamp et al., 1990); intra and interassay CV (n = 5 assays) were 9 and 17%, respectively. Plasma concentrations of glucose were quantified with an enzymatic colorimetric procedure (Thermo DMA, Louisville, Colorado); intra and interassay CV (n = 10 assays) were 3 and 6%, respectively. Concentrations of insulin in plasma were quantified with a solid phase RIA for human insulin (Coat-A-Count Insulin kit, Diagnostic Products Corp., Los Angeles, CA; Bossis et al., 1999) with bovine pancreatic insulin as the standard (Sigma Chemical Co., St. Louis, MO). Intra and interassay CV (n = 5 assays) were 5 and 18%, respectively. Concentrations of progesterone in plasma were quantified with a solid phase RIA (Coat-A-Count Progesterone kit. Diagnostic Products Corp.; Vizcarra et al., 1997) in one assay, and the intraassay CV was 16%.

Statistical Analyses

Body weight, ADG and BCS of the cows were analyzed as a completely randomized design with a 2 x 2 factorial treatment structure using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). The model included WG, bST, and the interaction. Body weight and ADG of the calves were analyzed as a completely randomized design with a 2 x 2 factorial treatment structure using the GLM procedure of SAS. The model included WG of the dam, bST, calf sex and the interactions. Interactions that were non-significant ($P > 0.30$) were eliminated from the final models.

Plasma concentrations of IGF-I, glucose and insulin were analyzed as a completely randomized design with a 2 x 2 factorial treatment structure using the MIXED procedure of SAS to evaluate repeated measurements of cows. Six covariance structures (variance component, compound symmetry, Huynh-Feldt, first-order autoregressive, Toeplitz and unstructured) were examined to select the best one according to the goodness of fit statistic. Variance component for all analyses were estimated using the restricted maximum-likelihood method. The covariance structure with the best goodness of fit statistics for glucose data was the first-order autoregressive, and for hormones the unstructured was selected. The Kenward-Roger procedure was used to determine the denominator degrees of freedom. The statistical model included WG, bST, day, block (laboratory assays), and the interactions. Block was considered to be random and all others effects in the model were considered fixed. All interactions among WG, bST and day were included in the initial model and non-significant ($P > 0.30$) interactions were eliminated from the final model. Data are expressed as least square means ± SE. When effects were significant, LSM were compared using LSD (pdiff option of SAS).

To describe the linear relationship among response variables PROC CORR from SAS was used. Concentrations of hormones and glucose before and after bST treatment were averaged for comparisons. Concentrations of hormones and glucose in plasma before bST treatment included d 24 to 31 post partum, and after bST treatment included d 34 to 59 post partum.

The percentage of cows with luteal activity by 59 d after parturition was analyzed as a completely randomized design with a 2 x 2 factorial treatment structure using GLIMMIX procedure of SAS.

RESULTS

Cows assigned to the four treatment combinations had similar ($P = 0.82$) BCS at calving (4.6 ± 0.1). At the end of the dietary treatment (d 59) H cows had greater ($P < 0.01$) BCS compared with the M cows (5.1 ± 0.09 vs. 4.4 ± 0.09, respectively; Table 1).

Body weight was similar ($P > 0.21$) for cows on the four treatment combinations before the first bST treatment. Cows on H had greater ($P < 0.01$) BW compared with M cows at the end of the dietary treatment (516 vs. 437 ± 11 kg, respectively; Table 1). Weight gain did not influence ($P = 0.11$) the ADG response to bST. High weight gain cows had greater ($P < 0.01$) ADG compared with M cows (1.43 vs. -0.16 ± 0.11 kg, respectively). Cows treated with bST had greater ($P = 0.01$) ADG compared with C cows (0.83 vs. 0.66 ± 0.11 kg, respectively).

Concentrations of IGF-I in plasma were greater ($P = 0.02$) in H compared with M cows (42.3 vs. 30.5 ± 3.7 ng/mL, respectively) before treatment with bST (Figure 1). After bST treatment, there was a WG x bST x day effect ($P < 0.01$) for plasma concentrations of IGF-I. Concentrations of IGF-I were greater ($P < 0.01$) on 3, 7 and 10 d, after the first bST treatment in HbST cows compared with MbST, MC and HC cows. Similarly, plasma concentrations of IGF-I were greater ($P < 0.001$) on 3, 7 and 10 d, after the second bST treatment in HbST compared with MbST, MC and HC cows. Concentrations of glucose in plasma were greater ($P = 0.06$) in H compared with M cows (70.0 vs 65.8 ± 1.5 mg/dL, respectively) before bST treatment during d 24 and 31 post

partum (Figure 2). After treatment with bST, there was a WG x bST effect ($P = 0.008$) on plasma concentrations of glucose. Concentrations of glucose in plasma were greater ($P < 0.005$) in HbST (72.4 ± 1.5 mg/dL) compared with HC, MC, and MbST cows (68.2 ± 1.6, 65.7 ± 1.5 and 64.2 ± 1.6 mg/dL, respectively). Plasma concentrations of IGF-I after the first bST treatment were positively correlated with glucose after the first bST treatment (r = 0.37, $P = 0.03$; Table 2).

Concentrations of insulin in plasma were greater ($P = 0.04$) in H (1.57 ± 0.34 ng/mL) compared with the M cows (1.34 ± 0.34 ng/mL) before bST treatment (Figure 3). After treatment with bST, there was a WG x day effect ($P < 0.001$) on plasma insulin. Concentrations of insulin in plasma were greater ($P < 0.01$) in H (1.74 ± 0.35 ng/mL) compared with M cows (1.25 ± 0.35 ng/mL) on d 34 and from d 45 to d 59. There was a tendency ($P = 0.07$) for a bST x day effect. Cows treated with bST tended to have greater concentrations of insulin in plasma compared with C cows on d 34. Plasma concentrations of insulin before and after the first bST treatment were positively correlated with IGF-I before the first bST treatment (r = 0.39, $P = 0.02$ and r = 0.44, $P = 0.01$, respectively; Table 2).

Body condition score at calving and at the end of the treatment were positively correlated with plasma concentrations of IGF-I before the first bST treatment (r = 0.37, $P = 0.02$ and r = 0.41, $P = 0.01$, respectively; Table 2). Body condition score at the end of dietary treatment was positively correlated with plasma concentrations of IGF-I, insulin, and glucose after the first bST treatment (r = 0.42, $P = 0.009$; r = 0.38, $P = 0.02$; and r = 0.51, $P < 0.001$, respectively; Table 2)

Weight gain and treatment with bST did not influence ($P = 0.99$) the percentage of cows with luteal activity by 59 d after parturition, and the subsequent calving interval was not influenced ($P \geq 0.33$) by treatment (Table 3).

There was not a WG x bST effect ($P > 0.30$) on average daily gain and BW of the calves (Table 3). Treatment with bST increased ADG of calves at 60 d ($P = 0.03$) and 140 d ($P = 0.06$) of age. Treatment with bST increased ($P = 0.02$) BW of calves at 60 d of age. Weight of calves at 220 d of age was not influenced ($P = 0.15$) by treatment with bST during early lactation. Cows in H treatment during early lactation had heavier calves at 60 d ($P < 0.01$), 140 d ($P < 0.01$), and 220 d ($P = 0.03$) of age.

DISCUSSION

Body weight and BCS of cows with H weight gain increased during the 59 d of treatment. Cows on H gain had a 42 kg increase in BW and a 0.47 increase in BCS, whereas M cows had a 6 kg decrease in BW and 0.01 decrease in BCS. Consumption of a high energy diet before puberty (Yelich et al., 1995), during pregnancy (Lents et al., 2005), or after parturition (Perry et al., 1991a; Ciccioli et al., 2003; Rubio, 2005) increased BW and BCS in growing heifers and beef cows. Body condition score was positively correlated with concentrations of IGF-I, insulin, and glucose in plasma. In agreement, BCS was positively correlated with concentrations IGF-I and insulin in plasma of primiparous beef cows (Lalman et al., 2000) and pregnant beef cows (Lents et al., 2005), and changes in plasma insulin usually correspond to similar changes in plasma concentrations of glucose (Richards et al., 1989).

Weight gain influenced plasma concentrations of IGF-I before bST treatment. High weight gain cows had greater concentrations of IGF-I in plasma compared with M

cows. Similarly, greater nutrient intake increased concentrations of IGF-I in plasma of beef heifers (Yelich et al., 1996; Bossis et al., 1999; Armstrong et al., 2001), and primiparous (Lalman et al., 2000; Ciccioli et al, 2003; Rubio, 2005) and multiparous beef cows (Richards et al., 1991). An increase in BW and BCS has been associated with greater IGF-I in crossbred cows (Roberts et al., 2005). After bST treatment, there was a weight gain x bST x day effect on IGF-I. High weight gain cows treated with bST had greater concentrations of IGF-I in plasma for 10 d after treatment with bST compared with cows on the other three treatments. Treatment of dairy heifers (Radcliff et al., 2004), dairy cows (Bilby et al., 1999) and beef cows (Armstrong et al., 1995; Flores et al., 2008) with bST increases concentrations of IGF-I in plasma. Moreover, the increase in plasma concentrations of IGF-I after bST treatment was greater in well-fed compared with under-fed growing beef cattle (Rausch et al., 2002). Similarly, plasma concentrations of IGF-I after bST treatment were greater in Holstein heifers on a high-gain diet compared with heifers on a low-gain diet (Radcliff et al., 2004), the greater IGF-I in plasma of heifers with greater nutrient intake could be a consequence of increased growth hormone receptors (GHR) in the liver. Decreased nutrient intake uncouples the GH-IGF-I axis (Thissen et al., 1994; Buttler et al., 2003). Dietary restriction results in increased GH, suppressed GHR in the liver, and decreased IGF-I secretion (Thissen et al., 1994). Reduced numbers of GHR in the liver is probably due to an insulin-dependent down regulation of receptors (Thissen et al., 1994; Kobayashi et al., 1999; Butler et al., 2003). Hepatic GHR and plasma IGF-I are positively correlated with nutrient intake (Donaghy and Baxter, 1996). Greater weight gain of beef cows in the present experiment increased BW and BCS and may have increased hepatic GHR and IGF-I. Infusion of

insulin to postpartum dairy cows, increased IGF-I mRNA and hepatic expression of GHR-1A, which was associated with a 3- to 6- fold increase in IGF-I and a 8- fold increase in insulin compared with controls (Butler et al., 2003). Increased plasma concentrations of insulin in high weight gain cows may have enhanced liver sensitivity to bST as well as IGF-I gene expression in the liver of these cows, and consequently increased plasma concentrations of IGF-I.

Concentrations of glucose in plasma were greater in H compared with M cows before bST treatment. In agreement with our results, heifers fed for high or moderate gain had greater plasma glucose than heifers fed to maintain BW (Yelich et al., 1995). Primiparous lactating beef cows on a high gain diet also had greater plasma glucose compared with those on a moderate gain (Ciccioli et al., 2003). Treatment with bST increased concentrations of glucose in plasma in high weight gain cows compared with other cows. Treatment with bST increased concentrations of glucose in plasma before calving but did not influenced glucose on early postpartum dairy cows (Gulay et al., 2004). Concentrations of glucose in serum (on d 68 post partum) of beef cows were not influenced by bST treatment every 14 d starting on d 28 post partum (Armstrong et al., 1995). Glucose metabolism is stimulated by bST treatment in lactating cows; glucose oxidation is reduced, gluconeogenesis in the liver is increased and insulin dependent glucose uptake by the cells is reduced (Bauman and Vernon, 1993). Altered metabolism, as well as nutrient availability for the high weight gain cows, may have accounted for the increase in concentrations of glucose in plasma after bST treatment.

Concentrations of insulin in plasma were greater for cows on a high weight gain compared with moderate cows before and after bST treatment. Before treatment with

bST, concentrations of glucose in plasma were greater in H cows, which probably stimulated greater secretion of insulin in plasma to regulate glucose. In agreement, plasma concentrations of insulin were also greater in beef heifers (Yelich et al., 1995) and primiparous beef cows (Lalman et al., 2000; Ciccioli et al., 2003; Rubio, 2005) fed a high gain diet compared with a maintenance or low BW gain diet. Treatment with bST increased concentrations of insulin in plasma of nonlactating nonpregnant dairy cows (Bilby et al., 2004). Treatment with low doses of bST, increased concentrations of insulin in plasma before parturition, but not during the early post partum period in dairy cows (Gulay et al., 2004). Similarly, concentrations of insulin in serum did not changed by d 68 post partum in beef cows treated every 2 wk with bST starting on d 28 post partum (Armstrong et al., 1995). Although concentrations of glucose in plasma were increased in high weight gain cows treated with bST, the expected increase in insulin was not observed in this study. It is probable that responsiveness of the pancreas to concentrations of glucose in plasma during early post partum is limited or that the sampling frequency was not adequate to detect changes associated with bST treatment.

Concentrations of IGF-I in plasma were positively correlated with concentrations of insulin and glucose in plasma after bST treatment. In agreement, concentrations of IGF-I in plasma were positively correlated with concentrations of insulin and glucose in plasma of primiparous beef cows (Ciccioli et al., 2003). However, acute injections of insulin (twice a day for 5 d) had no effect on plasma concentrations of IGF-I in beef cows (Simpson et al., 1994)

Luteal activity by d 59 post partum, and the subsequent calving interval, were not influenced by treatment, indicating no effect of treatment on reproductive functions.

Similarly, greater nutrient intake had no effect on duration of the postpartum anestrous interval in beef cows (Stagg et al., 1998). In contrast, a shorter interval to the first postpartum estrus (Ciccioli et al., 2003) and earlier resumption of luteal activity (Rubio, 2005) were observed in primiparous beef cows that had greater nutrient intake for 70 or 65 d (respectively) after calving, compared with cows with less nutrient intake. Similar cows with similar gain did not exhibit estrus until 90 to 120 d after calving (Ciccioli et al., 2003). In the present study, cows were on high weight gain for only 57 ± 1 d and the shorter treatment may have been inadequate to influence ovarian function. In addition, cows were treated with bST during the early postpartum period and treatment at 70 to 80 d after calving may have had a different effect on ovarian function. It is established that IGF-I stimulates ovarian function and amplifies gonadotropin action on steroidogenesis and follicle growth (Spicer et al., 1993; Spicer and Echternkamp, 1995; Lucy, 2000). Follicular size was influenced by nutritional treatment in primiparous beef cows on d 75 but not 56 post partum (Rubio, 2005). Maximum size of dominant follicles at d 56 post partum was similar in high BW gain cows compared with cows fed to maintain BW, although concentrations of IGF-I and insulin in plasma were increased (Rubio, 2005). Similarly, in the present study, concentrations of IGF-I in plasma were increased in the high weight gain cows treated with bST. The lack of effect of bST treatment on reproduction could be related to days after calving at treatment and/or duration of treatment. The physiological status of dairy cows affects the fertility response to bST treatment. Lactating dairy cows treated with bST had increased IGF-I concentrations in plasma and increased pregnancy rate after timed AI (TAI; Thatcher et al., 2006). Pregnancy rate in dry dairy cows treated with bST and TAI was decreased, although they

had greater concentrations of IGF-I compared with controls (Bilby et al., 2004). Concentrations of IGF-I in plasma of lactating cows are less than in non-lactating cows, which may influence the pregnancy response to bST treatment (Thatcher et al., 2006).

Differences in BCS at calving, amount of energy intake, duration of the feeding period, and other factors, may account for variations among different studies. This study contributes to the understanding of the influence of nutrition and bST on endocrine response of young beef cows and calf growth.

Postpartum weight gain of cows affected BW and ADG of the calves. High weight gain during early lactation increased BW and ADG of calves at d 60 and adjusted 220-d weaning weights. Similar to previous studies, calves suckling cows gaining 0.90 kg/d had increased BW at the end of the nutritional treatment and at weaning (Spitzer et al., 1995; Ciccioli et al., 2003). Greater energy intake in early lactation increases milk yield of beef cows (Perry et al., 1991a; Martson et al., 1995), and calf weight at weaning is highly related to milk yield (Neville, 1962; Totusek et al., 1973; Martson et al., 1995). Variability in weaning weight due to milk yield of the dam was 66% (Neville, 1962) and 60% (Rutledge et al., 1971) in Hereford calves. In addition, calves suckling high weight gain cows in the present study could consume some of the dam's feed which may have increased their weight gain. Treatment of cows with bST increased ADG and BW of calves at the end of the treatment. Treatment of lactating cows with bST increases nutrients available for the mammary gland and decreases synthesis of adipose tissue (Bauman, 1992). Additionally, bST treatment increases concentrations of IGF-I in plasma (Bilby et al., 1999) which mediates an increase in cell proliferation within the mammary gland. The increase in cell proliferation in addition to the increase in nutrient

availability for the gland promotes milk production (Bauman and Vernon, 1993). Similar to our results, beef cows treated with bST after 100 d post partum had increased milk yield, and greater ADG and weaning weight of calves (Armstrong et al., 1995). Treatment with bST during early lactation has a marginal effect on milk yield of dairy (Bauman and Vernon, 1993; Gulay et al., 2004) and beef cows (Armstrong et al., 1995). Weaning weights of calves were not influenced when treatment of beef cows with bST commenced on d 28 post partum (Armstrong et al., 1995). In our study, weaning weights of the calves tended to be 8 kg greater for cows that received bST, on d 31 and d 45 after calving, compared with control cows. Armstrong et al. (1995) found an increase in calf weaning weights of 15 to 20 kg, when treatments with bST (500 mg, biweekly) started after 100 d after calving.

IMPLICATIONS

Body weight and BCS of young beef cows are related to performance. Greater weight gain and treatment of postpartum beef cows with bST increased plasma concentrations of IGF-I. Cows that gained more than 0.4 kg/d of BW had greater concentrations of IGF-I and glucose in plasma after treatment with bST compared with cows that gained less than 0.4 kg/d of BW. Increased weight gain of cows during the first 59 d post partum, and treatment with bST on d 31 and d 45 post partum, did not alter the interval from calving to the onset of ovarian luteal activity or the calving interval. Greater weight gain and treatment of the cows with bST increased weigh gain and weaning weight of calves.

Table 1. Effect of weight gain (WG) and bST treatment on BCS and body weight (BW) of young lactating beef cows [1]

	Treatments[2]					*P* value		
Item	MC	MbST	HC	HbST	SE	WG	bST	INT[3]
Cows, n	10	8	9	10	-	-	-	-
BCS at calving	4.5	4.6	4.6	4.7	0.1	0.45	0.64	0.82
BCS 24 d post partum	4.5	4.4	4.7	4.7	0.1	0.10	0.91	0.98
BCS 59 d post partum	4.4	4.4	5.1	5.2	0.1	< 0.01	0.65	0.52
BW 24 d post partum, kg	439	447	457	474	18	0.21	0.81	0.49
BW 59 d post partum, kg	431	444	512	519	44	< 0.01	0.52	0.82
ADG, during 24 to 59 d post partum kg	-0.24	-0.09	1.09	1.76	0.16	< 0.01	0.01	0.11

[1]Body condition scores are: 1 = emaciated and 9 = obese (Wagner et al., 1988).

[2]MC = moderate weight gain, saline; MbST = moderate weight gain, bST; HC = high weight gain, saline; HbST = high weight gain, bST.

[3]INT = interaction between WG and bST.

Table 2. Correlation coefficients (r/*P* value), among BCS at calving, BCS at 59 d post partum, IGF-I, insulin and glucose in plasma before and after the first bST treatment in lactating beef cows (n = 37)[1]

	BCS at Calving	BCS d 59	IGF-I before	IGF-I after	Insulin before	Insulin after	Glucose before	Glucose after
BCS at Calving		0.43	0.37	0.16	0.07	0.11	0.21	0.21
		0.01	0.02	0.34	0.68	0.52	0.21	0.22
BCS d 59			0.41	0.42	0.17	0.38	0.23	0.51
			0.01	0.009	0.33	0.02	0.17	0.001
IGF-I before				0.28	0.39	0.44	0.25	0.23
				0.09	0.02	0.01	0.13	0.17
IGF-I after					0.12	0.18	0.18	0.37
					0.49	0.29	0.28	0.03
Insulin before						0.87	-0.03	-0.11
						<.0001	0.86	0.51
Insulin after							-0.16	-0.08
							0.34	0.63
Glucose before								0.77
								<.0001

[1] Concentrations of hormones and glucose in plasma before bST treatment include d 24 to 31 post partum, and after bST treatment include d 34 to 59 post partum.

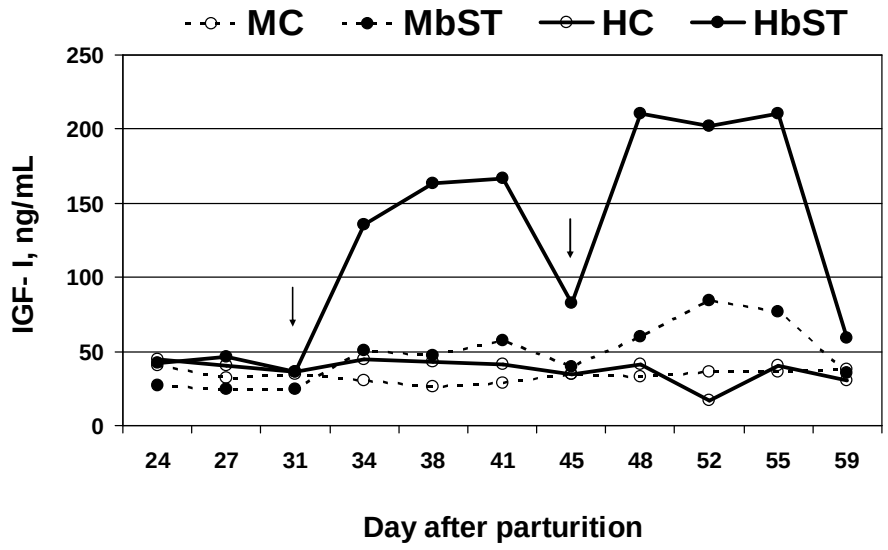

Figure 1. Least squares mean concentrations of IGF-I in plasma from 24 d to 59 d after parturition of lactating beef cows with Moderate (M, n = 18) or High (H, n = 19) weight gain and treated with bST or saline. Lines with open or solid circles represent cows that received saline (C, n = 19) or bST (n = 18) injection (250 mg), respectively. Arrows indicate bST injections. Standard errors averaged over days was 13.4.

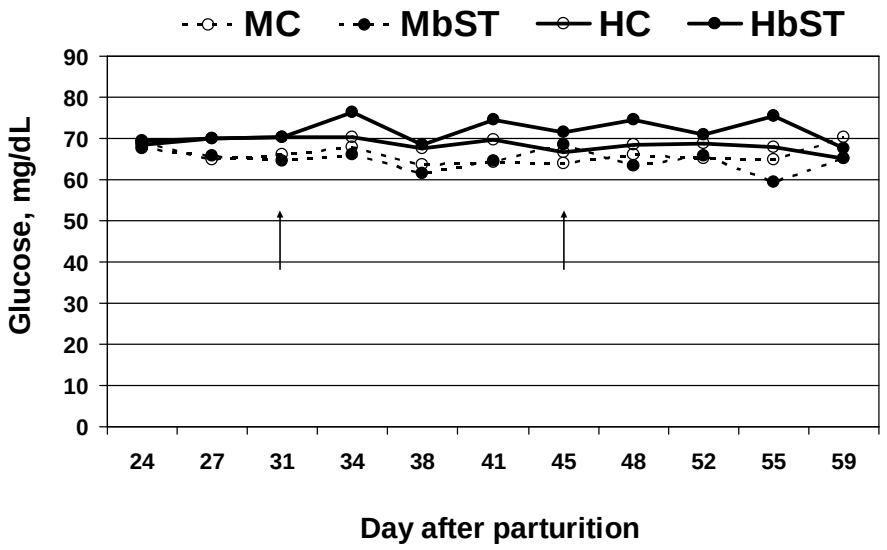

Figure 2. Least squares mean concentrations of glucose in plasma from 24 d to 59 d after parturition of lactating beef cows with Moderate (M, n = 18) or High (H, n = 19) weight gain treated with bST or saline. Lines with open or solid circles represent cows that received saline (C, n = 19) or bST (n = 18) injection (250 mg), respectively. Arrows indicate bST injections. Standard errors averaged over days was 2.8.

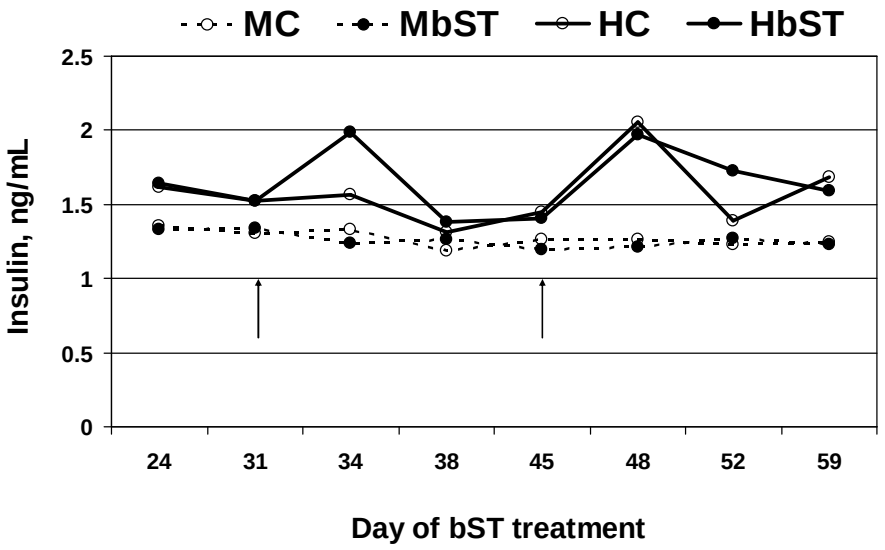

Figure 3. Least squares mean concentrations of insulin in plasma from 24 d to 59 d after parturition of lactating beef cows with Moderate (M, n = 18) or High (H, n = 19) weight gain treated with bST or saline. Lines with open or solid circles represent cows that received saline (C, n = 19) or bST (n = 18) injection (250 mg), respectively. Arrows indicate bST injections. Standard errors averaged over days was 0.4.

Table 3. Effect of weight gain (WG) and bST treatment on luteal activity (LA), calving interval, and average daily gain (ADG) and body weight (BW) of the calves from young lactating beef cows

	Treatments[2]					*P* value		
Item	MC	MbST	HC	HbST	SE	WG	bST	INT[3]
Cows, n	10	8	9	10	-	-	-	-
LA 59 d post partum, %	90	75	100	80		0.99	0.99	0.99
Calving interval, d[1]	357	358	362	352	5	0.88	0.44	0.33
Calves, n	9	7	8	10	-	-	-	-
ADG at:								
60 d of age, kg	0.82	0.90	1.06	1.16	0.12	< 0.01	0.03	0.78
140 d of age, kg	0.84	0.90	0.95	1.02	0.10	< 0.01	0.06	0.95
220 d of age, kg	0.78	0.81	0.83	0.88	0.07	0.01	0.15	0.73
BW at:								
60 d of age, kg	85	87	97	105	3	< 0.01	0.02	0.32
140 d of age, kg	153	160	167	178	5	< 0.01	0.10	0.63
220 d of age, kg	206	210	217	229	6	0.03	0.19	0.53

[1]Subsequent calving interval.

[2]MC = moderate weight gain, saline; MbST = moderate weight gain, bST; HC = high weight gain, saline; HbST = high weight gain, bST.

[3]INT = interaction between WG and bST.

CHAPTER IV

MAINTENANCE ENERGY REQUIREMENTS OF GESTATING BEEF COWS AND RELATIONSHIP WITH COW AND CALF PERFORMANCE, METABOLIC HORMONES, AND FUNCTIONAL PROTEINS

ABSTRACT: Angus x Hereford nonlactating, spring-calving cows were used to determine variation in maintenance energy requirements (MR), to evaluate the relationship among MR and cow and calf performance, plasma concentrations of IGF-I, T_4, glucose, insulin and ruminal temperature, and to describe the LM proteome and evaluate protein abundance in cows with different MR. Cows (4 to 7 yr of age) with a BCS of 5.0 ± 0.2, and BW of 582 ± 37 kg, in the second to third trimester of gestation, were studied in 3 yr (yr 1, n = 23; yr 2, n = 32; yr 3, n = 38). Cows were individually fed a complete diet in amounts to meet predicted MR (Level 1 Model, NRC 1996), and daily FI was adjusted weekly until constant BW was achieved for at least 21 d (maintenance). Cows were classified based on MR as low (> 0.5 SD less than mean, LMR), moderate (± 0.5 SD of mean, MMR) or high (> 0.5 SD more than mean, HMR) MR. Blood samples were taken at maintenance and at 2 mo post partum on yr 2. Muscle biopsies were taken from LMR and HMR at maintenance in yr 2 and 3. Proteins from LM were separated by two-dimensional, difference gel electrophoresis and abundance was quantified and compared. The greatest differences in MR between cows were 29,

24, and 25% in yr 1, 2 and 3, respectively. Daily MR (NE_m, $Kcal \cdot BW^{-0.75} \cdot d^{-1}$) averaged 89.2 ± 6.3, 93.0 ± 4.9, and 90.4 ± 4.6, in yr 1, 2 and 3, respectively. Postpartum BW and BCS, calves birth and weaning weights, resumption of luteal activity, plasma concentrations of hormones and ruminal temperature were not influenced by MR of the cows. However, MR was negatively correlated with concentrations of IGF-I in plasma (r = -0.38; *P* = 0.05) and tended to be positively correlated with T_4 in plasma (r = 0.31; *P* = 0.12) at 2 mo post partum. A total of 103 proteins were isolated, 52 gene products were identified, of which many (33%) participated in metabolism. Protein abundance tended (*P* = 0.11) to be greater in HMR for cofilin-2. Greater abundance of cofilin-2 in HMR cows may have application as a biomarker for MR. Identification of biomarkers for MR will allow selection of more efficient cows, that consume less feed and produce calves with similar weaning weights. Productive cows that require less feed for maintenance will improve efficiency of production and enhance the sustainability of the environment.

Key words: beef cattle, IGF-I, maintenance energy requirements, proteomics.

INTRODUCTION

The efficiency of beef production could be improved by decreasing the energy required for maintenance of cows. Maintenance energy requirement (MR) of cow is the greatest variable cost in beef production. Approximately 70% of the total annually energy required for beef cows is due to MR, which is independent of cow type (Ferrell and Jenkins, 1984). Differences in MR within and between breeds and types have been recognized (Ferrell and Jenkins, 1984; DiCostanzo et al., 1990; Derno et al., 2005). The CV of MR in beef cattle ranges from 5 to 35%, indicating important differences within breeds (Johnson et al., 2003). In addition, heritability of MR is moderate (Hotovy et al.,

1991). Identification and selection of beef cows with lesser MR, that wean a calf each year, should decrease inputs, increase production efficiency, and enhance the sustainability of the environment. Methods to estimate MR are feeding trials, comparative slaughter and calorimetric methods (NRC, 1996); these methods are expensive and/or time consuming.

Identification of biomarkers for low MR of beef cows is essential to increase progress in selecting for this trait. Metabolic hormones could be regulators of biological processes that are responsible for variation in MR. Nutrient intake influences concentrations of IGF-I, insulin, and thyroxine (T_4) in plasma of beef cows (Ciccioli et al., 2003; Lents et al., 2005). Rectal temperature has been associated with MR in beef steers (Derno et al., 2005) and mice (Kgwatalala et al., 2004). Skeletal muscle requires an important proportion of energy expenditure for muscle contraction and protein turnover (Webster, 1980; Tess et al., 1984; Lobley, 2002). Mitochondrial efficiency was increased in *Longissimus dorsi* of more efficient steers (Kolath et al., 2006). Radioimmunoassay, rumen temperature boluses, and proteomic analyses are techniques to investigate biomarkers for MR. Rumen boluses to record body temperature are practical and have minimal impact on animal behavior. Proteomic analyses describe protein expression at given times and conditions, and biomarkers may be discovered with the use of proteomics without previous knowledge of the proteins (Lippolis and Reinhardt, 2008). Concentrations of hormones in plasma, body temperature, and muscle protein expression in mature beef cows with different MR have not been determined. Therefore, objectives of this study were: (a) to determine variation in MR of mature, nonlactating, beef cows during mid- to late-gestation, (b) to evaluate relationships

between MR, cow performance, and postnatal calf growth (c) to evaluate relationships among MR and plasma concentrations of IGF-I, thyroxine, glucose, insulin, and ruminal temperature, (d) and to describe the proteome of *Longissimus dorsi* and evaluate protein abundance and potential biomarkers in mature beef cows with different MR.

MATERIALS AND METHODS

Animal management and estimation of maintenance energy requirements

The Oklahoma State University Animal Care and Use Committee approved all the experimental procedures used in this study. Spring-calving Angus x Hereford cows were utilized during 3 yr to determine the influence of MR on physiological functions. Estrous cycles were synchronized and cows were AI to a single sire during 3 wk each year. Calves were weaned at 7 mo of age. Nonlactating pregnant cows (age = 4 to 7 yr) were 6 to 8 mo of gestation during evaluation of MR (November to January) in yr 1 (BW = 595 ± 24 kg; BCS = 5.1 ± 0.2; n = 23). Cows in yr 2 (BW = 576 ± 47 kg; BCS = 5.0 ± 0.2; n = 32) and yr 3 (BW = 569 ± 44 kg; BCS = 4.9 ± 0.3; n = 37) were 5 to 7 mo of gestation during evaluation of MR (October to December). Calf growth and cow performance were evaluated in yr 1 and 2, hormones in plasma and ruminal temperature were evaluated in yr 2, and the abundance of proteins in *Longissimus dorsi* were determined in yr 2 and 3.

Maintenance energy requirement was defined as the amount of dietary energy intake that resulted in no net loss or gain of energy from body tissues (NRC, 1996), and consequently BW and BCS of the cow remained constant. Fetal weight increase during determination of MR represented 2 to 3% of maternal BW, was similar for all cows, and was not considered. Cows were individually fed at 0730 h once a day. The diet (as fed)

was composed of rolled corn (38%), alfalfa pellets (35%), cottonseed hulls (21%), soybean meal (4%), cane molasses (3%), salt (0.2%) and vitamin A, with a calculated (as fed) CP = 11.2% and NE_m = 1.44 Mcal/kg. Samples of the ration were taken twice a week to prepare a composite for analyses (Dairy One, Inc., Ithaca, NY). The analyzed (as fed) CP and NE_m content of the ration were 13.46% and 1.45 Mcal/kg, respectively in yr 1, and 14.1% and 1.45 Mcal/kg, respectively in yr 2, and 13.0% and 1.41 Mcal/kg, respectively in yr 3.

Body weights were recorded weekly after feed and water deprivation for 23 and 17 h, respectively. Body condition scores (1 = emaciate, and 9 = obese; Wagner et al., 1988) were determined at the beginning and end of the trial to confirm constant body composition. Cows were fed a maintenance diet based on NRC recommendations, using the "describe animal" screens in Level 1 Model (NRC, 1996), for 21 d. The initial BW was determined after 7 d when the trial started. After 14 d the daily diet was adjusted every 7 d to maintain constant BW. When the cumulative increase in BW of a cow was greater than 9.07 kg for 2 wk, the ration was reduced by 45 kg feed/d compared with the previous week. Cows with cumulative decrease in BW greater than 9.07 kg for 2 wk, received an additional 0.45 kg of feed/d compared with the previous week. The MR of cows was determined during the same days when all cows had a constant body weight for at least 21 d. Cows consumed the total diet that was offered. Cows had ad libitum access to mineral mix (46.1% NaCl, 50.0% dicalcium phosphate, 0.4% copper sulfate, 0.5% zinc oxide and 3.0% mineral oil) and water. Constant BW of cows was determined with regression analysis using PROC REG (SAS Inst., Inc., Cary, NC). Cows with a

significant ($P < 0.10$) linear regression of BW over days, indicating BW gain or loss, were eliminated from analyses.

Maintenance energy requirement (NE_m) of each cow was calculated as the dietary energy required to maintain constant BW for 21 d (yr 1 and 3), and 28 d (yr 2), expressed as $Kcal \cdot BW^{-0.75} \cdot d^{-1}$. Mean BW during the period of constant BW and the mean daily energy (NE_m) consumed during that period were used to calculate MR. Cows were classified into: low (> 0.5 SD less than mean, LMR), medium (± 0.5 SD of mean, MMR) or high (> 0.5 SD more than mean, HMR) MR groups.

After determination of MR, cows were maintained as a group, grazed native prairie pasture (*Andropogon scoparius, Andropogon gerardii*), and received protein supplementation according to their physiological state and pasture availability. During the last trimester of gestation, cows received 1.40 kg/d of a 38% CP supplement. After parturition, supplementation was increased to 1.80 kg/d of the same supplement, for 2 mo, and cows had prairie hay ad libitum. In yr 2, ruminal temperature was recorded from 10 d before to 7 d after parturition cows. Cows were maintained in a pen (60 x 80 m), and fed 1.8 kg/d of a 38% CP supplement, with water and prairie hay ad libitum. Calves remained with dams continuously.

Body weight and BCS

Body weight of cows and calves, and BCS of cows were recorded after 17 h without feed and water at 2 and 6 mo after calving and at weaning. Calves were weaned at 7.1 ± 0.3 mo and 6.9 ± 0.2 mo of age in yr 1 and 2, respectively.

Ruminal temperature records

Boluses (SmartStock®, LLC, Pawnee, OK) were orally placed into the rumen of each cow using a custom balling gun, at 7.0 ± 0.2 month of gestation in yr 2. The ruminal bolus data collection system (SmartStock®, LLC) consisted of four components: (a) radio frequency ruminal temperature sensor bolus (8.25 cm x 3.17 cm; 114 g), (b) an antenna within the cow pen for data collection, (c) a receiver antenna within 100 m of the antenna for data collection, and (d) a personal computer with software for data collection. Date, time, cow identification and ruminal temperature (every 15 min) were transmitted by radiotelemetry and stored in the computer for analyses.

Blood samples, hormones and assays

Blood was collected at 0730 (after deprived from water and feed for at least 18 h), 1100 and 1430 h (at 2 and 5.5 h after feeding) on 3 different days in yr 2. The first day of sampling occurred after cows were fed to the predicted MR level (NRC Level 1 Model) for 2 wk (d 14 of the trial). The second day of sampling occurred after cows were fed their actual maintenance diet for 28 d (d 49 of the trial). The third day of sampling occurred at 2 mo after calving when cows were grazing native prairie grass pasture (d 189 after initiation of the trial). Blood samples were collected from caudal veins into vacutainer tubes containing EDTA, stored on ice, centrifuged at 2500 x g for 20 min at 4°C within 2 h after collection, and plasma was aspirated and stored at -20°C.

Commencing at 55 ± 10 (yr 1) and 35 ± 10 (yr 2) d after calving. Blood samples were taken for progesterone analyses twice a week for 3 wk or until luteal activity was confirmed. The criterion for luteal activity was that progesterone > 1 ng/mL for 2 or more sequential samples collected at 3 or 4 d intervals (Wettemann et al., 1972).

Concentrations of IGF-I in plasma were determined after acid ethanol extraction (16 h at 4 ºC) by RIA (Echternkamp et al., 1990). Samples collected on d 14 were analyzed in one assay, samples collected on d 49 were analyzed in a second assay and samples collected on d 189 were analyzed in a third assay. Intra and interassay CV (n = 3 assays) were 3 and 17%, respectively. Plasma concentrations of T_4 were quantified with a solid phase RIA for human T_4 (Coat-A-Count Total T_4 kit, Diagnostic Products Corp., Los Angeles, CA; Ciccioli et al., 2003). Samples from d 49 and 189 were analyzed in 4 assays per day of collection. The intra and interassay CV (n = 4 assays) were 8 and 5%, respectively. Plasma concentrations of glucose were quantified with an enzymatic colorimetric procedure (Thermo DMA, Louisville, CO). Samples from d 14, 49 and 189 were analyzed in 4 assays per day of collection. Intra and interassay CV (n = 12 assays) were 3 and 3%, respectively. Concentrations of insulin in plasma from d 49 and 189 were quantified with a solid phase RIA for human insulin (Coat-A-Count Insulin kit, Diagnostic Products Corp.; Bossis et al., 1999) with bovine pancreatic insulin as the standard (Sigma Chemical Co., St. Louis, MO). The intraassay CV (n = 1 assays) was 2.6%. Concentrations of progesterone in plasma were quantified with a solid phase RIA (Coat-A-Count Progesterone kit. Diagnostic Products Corp.; Vizcarra et al., 1997).

Muscle sample collection

Muscle samples were obtained from six LMR and six HMR cows in each of yr 2 and 3 after cows consumed their actual MR on d 28 (yr 2) and d 21 (yr 3). *Longissimus dorsi* biopsies (Winterholler, et al. 2008) were taken from the 12 cows each year. Using a sterile biopsy needle (7 mm i.d.). All the samples (400 mg of fresh tissue) were taken within 3 h, approximately 10 cm caudal to the last rib, and 8 cm lateral to the vertebrae.

Samples were immediately frozen in liquid nitrogen and stored at – 80ºC. Proteomic analyses were conducted in two replications with 6 pairs (LMR and HMR) of samples from the same yr in each experiment.

Extraction of muscle proteins

Frozen muscle samples (50 mg) were individually homogenized (Tissue tearor model 985370-395; Biospec Products Inc.; Bartlesville, OK) in 0.5 mL of 7 M urea, 2 M thiourea, and 4% CHAPS (3-[3-(cholomidopropyl)-dimethyl-ammonio]-1-propanesulphonate) on ice. Homogenized tissue was rocked (Multitube Rotator, Model N^{o} 4632, Barnstead Lab-Line, Melrose Park, IL) for 3 to 4 h at 4 °C. Extractions were ultrasonicated (Fisher sonic dismembrator model 300; Miami, OK) on ice, 2 times for 15 sec, with 1 min interval between. Then samples were centrifuged (Beckman microfuge E^{TM}; Fullerton, CA) at 15,000 x g for 7 min at 4 °C. Protein concentrations were estimated by a modified Bradford assay (Ramagli, 1999) and the concentration was 1.0 ± 0.1 µg /uL.

Protein labeling

Protein extractions (6 pairs of LMR and HMR) were labeled using fluorescent dyes (CyDyes^{TM}, GE Healthcare Bio Sciences, Amersham, Piscataway, NJ), following the manufacturer's instructions. The dyes used were Cy2, Cy3 and Cy5. Briefly, samples from LMR and HMR cows, within year, were randomly assigned to previously establish labeling reactions (Table 4). Protein labeling reactions were set up such that: a) both MR groups were equally represented by Cy3 or Cy5 dye to minimize possible variation due to the dye-protein binding, and b) a sample from each MR group was processed one after the other in the order they will be mixed for first dimension. Low MR or HMR protein

extractions were labeled with either Cy3 or Cy5 dye (50 µg of protein/400 pmol of dye) during 30 min at 4 °C. Then, L-lysine was added (10 mMol; 1uL/400 pmol of dye) for 10 min at 4 °C to sequester any possible unbound Cy-dye molecules in the sample. An internal pooled standard (300 µg of proteinin 300 uL) was prepared by pooling equal aliquots (25 µg in 25 uL) of all protein extractions, and labeled with Cy2 at the same time as LMR and HMR labeling reactions, as previously described. All labeling reactions and procedures were conducted in the dark.

Two-dimensional, fluorescent, difference gel electrophoresis (2-D DIGE)

The first dimension of isoelectric focusing was performed using a Multiphor II gel apparatus and Immobiline DryStrips (IPG strips, pH 4–7, 24 cm x 0.35 cm; GE Healthcare Bio Sciences) at 20 ºC. Each of the 6 DryStrips was rehydrated with labeled protein from one LMR cow, one HMR cow, and the internal pooled standard following arrangement in Table 4. Equal amounts of protein (50 µg in 50 µL) from each protein extraction and the internal pooled standard that were labeled with Cy3, Cy5 and Cy2 were mixed and added to 300 uL of rehydration buffer (6 M urea, 2 M thiourea, 2% w/v CHAPS, 50 mM dithiothreitol, 0.75% v/v IPG buffer pH 4-7). The IPG strip was rehydrated with the 450 µL mixture for 15 h under mineral oil. Isoelectric focusing was performed in 2 phases: 200 V for 6 h, and 3500 V for 28 h, for a total of 99.2 kVh. Focused IPG strips were stored overnight at – 20 °C.

The second dimension SDS-PAGE was performed using 12% acrylamide gels [1.5 M Tris-HCL pH 8.8, 12% T acrylamide/bisacrylamide, 0.1% w/v SDS, 0.05% w/v Ammonium persulfate (APS), 0.05% v/v tetramethylethylenediamine (TEMED)] casted in low fluorescence glass plates (gel size 24 x 20 x 1 cm^3). Prior to electrophoresis,

individual IPG strips were equilibrated in two steps (15 min each) using 10 mL of SDS equilibration buffer (6 M urea, 30% v/v glycerol, 2% w/v SDS and 50mM Tris pH 8.8) plus 1% w/v DTT for the first equilibration, or adding 2.5% w/v iodoacetamide for the second equilibration. Equilibrated strips and prestained protein standard (15 µL, 10 to 250 kDa, Bio-Rad laboratories, Inc., Richmond, CA) in filter paper were loaded on top of the casted separation gels, overlaid with 0.7% w/v agarose, and placed into a Ettan DALT six apparatus (GE Healthcare Bio Sciences) at 20 °C, using 1 x SDS running buffer (25 mM Tris, 192 mM glycin, 0.1% w/v SDS) in the lower chamber and 3 x SDS running buffer in the upper chamber. The second dimension was run at 15 mA/gel for the first 15 min followed by 30 mA/gel until the blue color from the protein standard front had run to the bottom of the gel.

Protein identification

An extra gel was run for protein identification. Unlabeled proteins from a pooled extraction from one LMR and one HMR cow were separated using the 2D procedure as previously described. However, a greater amount of protein (470 µg in 470 µL) was loaded into the IPG strip in order to be stained and visualized with coomassie blue after the 2 dimensional gel electrophoresis. Protein spots were numbered and excised using a plastic straw. Protein identification was performed at the Oklahoma State University recombinant DNA/Protein Core Facility. Excised spots were placed in 96-well plates (Microtiter 96-well plates, Thermo Scientific, Waltham, MA) and MALDI-TOF analysis was performed using a mass spectrometer [DE-PRO (Applied Biosystems) with reflector, CID module] after trypsin digestion. Data were matched to NCBI and SWISS PRO using Mascot Daemon software (Mascot 2.2, Matrix Science Ltd., London, UK;

http://www.matrixscience.com). The search criteria for peptide fingerprints was: digestion enzyme was trypsin, oxidation (M), propionamide (C), pyro-glu (N-term Q), peptide mass tolerance of 100 ppm, peptide mass charge 1+, maximum cleavage 1, and number of queries 18. Identification of a protein required the following criteria: MASCOT probabilistic scores above 50%, MASCOT probability-based MoWSe score of the top-ranked candidate(s) exceeded the threshold for a significant result and was notably greater than those of the next ranked candidate, consistent theoretical and experimental MW, and at least 5 matched peptides/protein.

Proteins with no clear identification after MALDI-TOF analysis and data base search, were further analyzed on the Orbitrap (Electrospray tandem MS = MS mass spectrometer [LTQ (ThermoFinnegan) with nano-LC (Eksigent) and infusion, microspray, and nanospray electrospray ion sources]). Data were processed in NCBI and SWISS PRO using SCAFFOLD software (V2.1.03 Proteome Software Inc., Portland, OR; http://www.proteomesoftware.com). Search criteria: tandem mass spectra were extracted using the extract msn utility (Bioworks 3.3.1, Thermo Fisher Scientific Inc., Miami, OK). All MS/MS samples were analyzed using Mascot (Mascot 2.2, Matrix Science Ltd.) and X! Tandem (V2007.01.01.1; The Global Proteome Machine Organization, Beavis Informatics Ltd, Winnipeg, Canada, www.thegpm.org). Mascot was set up to search the SwissProt New database (selected for Mammalia, 63,690 proteins) assuming the digestion enzyme trypsin. X! Tandem was set up to search the SwissProt New database (selected for all entries, 398,181 proteins) also assuming the digestion enzyme was trypsin. Mascot and X! Tandem were searched with a fragment ion mass tolerance of 0.80 Da and a parent ion tolerance of 10.0 ppm, S-

carbamoylmethylcysteine cyclization (N-terminus) of the n-terminus, oxidation (M), n-formylation of the n-terminus, acetylation of the n-terminus and acrylamide adduct of cysteine were specified in Mascot and X! Tandem. Identification of a protein required the following criteria: peptide probability greater than 95.0%, protein probability greater than 99.9%, and at least 2 identified peptides.

Images and proteomic data analyses

Fluorescence images of the 12 gels were acquired on a Typhoon trio scanner (GE Healthcare Bio Sciences) using parameters recommended by the manufacturer. Cy2, Cy3, and Cy5 images for each gel were scanned at 488 nm/520BP40, 532 nm/580BP30, and 633 nm/670BP30 excitation/emission wavelengths, respectively, at 100-µm resolution, thus obtaining a total of 36 images (6 x 3 x 2). Image analysis of the muscle proteins was performed using DeCyder^{TM} V5.0 (GE Healthcare Bio Sciences) following the manufacturer's recommendations. The differential in-gel analysis module (DIA) was used for intragel co-detection and normalization of samples and internal standard protein spots. Artifactual spots (dust and others) were filtered and removed. The biological variation analysis (BVA) module was used for intergel matching of internal standard and samples across all gels and performing comparative cross-gel statistical analyses of all spots based on standardized log abundance, permitting the detection of differentially expressed spots between LMR and HMR by ANOVA. Standardized log abundance for each spot was the logarithm (base 10, Log_{10}) of the normalized spot volume ratio (Cy3/Cy2 or Cy5/Cy2). The null hypothesis tested was: there is no change in the protein abundance of LMR and HMR groups analyzed. Matches and data quality of proteins of interest were manually checked.

Statistical analyses

Body weight and BCS of cows and calves, within year, were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). The statistical model included MR, calf sex and the interaction. Body weights of calves for yr 1 and yr 2 were combined and further evaluated using the MIXED procedure of SAS (SAS Inst., Inc.), with the model previously described including year as a random effect and all other effects were fixed. When effects were significant, LSM were compared using LSD (pdiff option of SAS).

Body weight and BCS of cows after the end of the trial were evaluated using the MIXED procedure of SAS (SAS Inst., Inc). The statistical model included MR, day after the end of the trial, calf sex and the interactions. Non-significant ($P > 0.30$) calf sex or interaction effects were deleted from the model. Six covariance structures (variance component, compound symmetry, Huynh-Feldt, first-order autoregressive, Toeplitz and unstructured) were examined to identify and use the best structure according to the goodness of fit statistic. Variance components for all analyses were estimated using the restricted maximum-likelihood method. The covariance structure with the best goodness of fit statistics for cow BW after the trial was compound symmetry. The Kenward-Roger procedure was used to determine the denominator degrees of freedom.

Days to resumption of luteal activity was analyzed using the GLM procedure of SAS (SAS Inst., Inc) with MR in the model. Concentrations of hormones and metabolites in plasma were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst., Inc). Hormone concentrations in plasma were analyzed within the day the samples were taken. Similarly to previous analyses, six

covariance structures were evaluated to select the appropriate structure. The covariance structure with the best goodness of fit was the first-order autoregressive in all cases, and was used for the analyses. The Kenward-Roger procedure was used to determine the denominator degrees of freedom. The statistical models, for IGF-I, T_4, insulin and glucose, included MR, hour, block (laboratory assay, if more than one) and the interactions. Block was a random effect and all others effects in the model were fixed. Those interactions that were non-significant ($P > 0.30$) were deleted from the final model. When effects were significant, LSM were compared using LSD (pdiff option of SAS).

Linear relationships among response variables were determined with PROC REG and PROC CORR of SAS (SAS Inst., Inc). A regression model to predict MR ($Kcal \cdot BW^{0.75} \cdot d^{-1}$) was built using PROC REG and the forward stepwise procedure. Insulin, IGF-I , T_4, glucose, BW and BCS were included if the associated slope was significant ($P < 0.15$) in the full model. Variables remained in the model if the slope was significant ($P < 0.3$) in the final model, Cp criterion was lower, and adjusted R^2 was increased by 5%.

Ruminal temperatures were analyzed using the MIXED procedure of SAS (SAS Inst., Inc). Similar to previous analyses, six covariance structures were evaluated to select the appropriate structure. The covariance structure with the best goodness of fit was the first-order autoregressive, and was used for the analyses. Mean daily ruminal temperatures were calculated for each cow (20 to 29 cows per day) for 3 to 7 d before parturition. The initial model included MR, day relative to parturition, and mean ambient temperature as a covariable. Day relative to parturition ($P = 0.50$) and mean ambient

temperature (P = 0.82) were deleted from the final model. When effects were significant, LSM were compared using LSD (pdiff option of SAS).

RESULTS

Durations of the trial when cows were fed a complete diet to determine MR were 9 wk in yr 1, 7 wk in yr 2, and 7 wk in yr 3. Cows maintained constant BW and BCS for 21 d in yr 1 (n = 20), 28 d in yr 2 (n = 27), and 21 d in yr 3 (n = 38). Daily ambient temperatures during the period when MR of cows was determined averaged 8, 2, and 2 °C in yr 1, 2, and 3, respectively. Maximum ambient temperatures during the period when MR of cows was determined averaged 26, 24, and 9 °C in yr 1, 2 and 3, respectively. Minimum ambient temperatures during the period when MR of cows was determined were -7, -13, and -3 °C in yr 1, 2, and 3, respectively (Mesonet, site Marena; www.mesonet.org).

Initial and final BW of the cows were 595 ± 24 kg and 604 ± 25 kg, respectively, in yr 1, and initial and final BCS of cows were 5.1 ± 0.2 and 5.1 ± 0.3, respectively in yr 1. In yr 2, initial and final BW of cows were 576 ± 47 kg and 569 ± 45, respectively, and initial and final BCS were 5.0 ± 0.2 and 5.2 ± 0.2, respectively. In yr 3, initial and final BW of cows were 565 ± 45 kg and 572 ± 43, respectively, and initial and final BCS were 4.9 ± 0.3 and 5.1 ± 0.3, respectively.

Daily MR (NE_m, $Kcal \cdot BW^{-0.75} \cdot d^{-1}$) averaged 89.2 ± 6.3, 93.0 ± 4.9, and 90.4 ± 4.6 in yr 1, yr 2, and yr 3, respectively. The difference between cows with the least and the greatest MR was 29% and the CV was 7% in yr 1 (Figure 4). The difference was 24% and the CV was 5 % in yr 2 (Figure 5). And the difference was 25% and the CV was 5 % in yr 3 (data not shown). The average MR in yr 1 was less (4.7 $Kcal \cdot BW^{-0.75} \cdot d^{-1}$) than the

NRC (Level 1 Model) estimated MR. In yr 2, average MR was greater (5.7 Kcal·$BW^{-0.75}$·d^{-1}) than the NRC (Level 1 Model) estimated MR. Cows in the LMR, MMR, and HMR groups differed ($P < 0.05$) in the actual amount of daily energy required to maintain constant BW and BCS in yr 1 and 2 (Table 5). Maintenance energy requirements were not influenced by age ($P \geq 0.45$) or BW ($P \geq 0.2$) of cows in yr 1 and yr 2 (Figure 6). Body weight changes of cows from parturition to weaning were not influenced ($P > 0.1$) by MR in yr 1 (Table 6). Body weight change, from the last day when cows were fed to maintenance, to one month after the end of the trial in yr 2, was influenced ($P = 0.02$) by MR of the cows. Body weight of the cows increased after the trial. The increase in BW of HMR cows was greater ($P = 0.006$) compared with the increase in BW of LMR cows, and the increase in BW of MMR was greater ($P = 0.05$) compared with the increase in BW of LMR cows. Body weight change from late gestation through weaning was not effected by MR x day or by MR in yr 1 ($P = 0.2$, $P = 0.6$, respectively; Figure 7) and or in yr 2 ($P = 0.29$, $P = 0.18$, respectively; Figure 8). Body weight decreased ($P < 0.001$) after parturition both years.

Body condition score at parturition was not influenced by MR in yr 1 ($P = 0.73$) or in yr 2 ($P = 0.17$; Table 6). In yr 1, BCS at weaning tended ($P = 0.08$) to be influenced by MR of the cows with the greater BCS for LMR. However, BCS at weaning was not influenced ($P = 0.27$) by MR in yr 2 (Table 6). Body condition score of cows from parturition through weaning was not effected by MR x day or by MR in yr 1 ($P = 0.31$, $P = 0.16$, respectively; Figure 9) or in yr 2 ($P = 0.38$, $P = 0.14$, respectively; Figure 10). Body condition score decreased ($P < 0.001$) after parturition in yr 1 and 2.

Weight of calves at birth and weaning was not influenced ($P > 0.59$) by MR of dams (Table 7).

Initiation of luteal activity occurred at less than 57 ± 9 d after calving for 95% of the cows in yr 1, before the first blood sample was collected. Resumption of luteal activity after calving in yr 2 was not effected ($P = 0.57$) by MR of the cows and averaged 48 ± 11 d (Table 7).

Concentrations of IGF-I in plasma were not effected ($P > 0.08$) by MR x hour at any time in yr 2. Concentrations of IGF-I in plasma were greater ($P < 0.001$) for MMR cows compared with LMR cows after 14 d consuming the predicted (NRC) MR diets in yr 2 (Figure 11), and tended ($P = 0.06$) to be greater in MMR cows compared with HMR cows, after 14 d consuming predicted (NRC) MR diets in yr 2. Concentrations of IGF-I in plasma were not influenced by MR at maintenance ($P = 0.15$) or at 2 mo after parturition (P = 0.11; Figure 12). Plasma concentrations of IGF-I were not influenced ($P = 0.4$) by hour after 14 d consuming predicted (NRC) MR diets (data not shown). Plasma concentrations of IGF-I were greater ($P = 0.02$) at 0730 and 1100 compared with 1430 h at maintenance (Table 8). Similarly, plasma concentrations of IGF-I were greater ($P \leq 0.01$) at 0730 and 1100 compared with 1430 h at 2 mo post partum (Table 8).

Concentrations of T_4 in plasma were not effected ($P > 0.54$) by the interaction MR x hour at any time in yr 2. Concentrations of T_4 in plasma tended to be influenced ($P = 0.08$) by MR at maintenance, with greater concentrations in HMR compared with MMR and LMR cows (Figure 12). Plasma concentrations of T_4 were not influenced ($P = 0.36$) by MR at 2 mo post partum. Plasma concentrations of T_4 were greater ($P \leq 0.01$) at 1100 and 1430 compared with those at 0730 h at maintenance (Table 8). Similarly, plasma

concentrations of T_4 were greater ($P < 0.001$) at 1100 and 1430 compared with those at 0730 h at 2 mo post partum (Table 8).

Concentrations of glucose in plasma were not effected ($P > 0.1$) by MR x hour or by the hour of sampling at any time in yr 2. Concentrations of glucose in plasma did not differ ($P = 0.18$) among MR treatments after 14 d consuming predicted (NRC) MR diets (Figure 11), at maintenance ($P = 0.93$), or at 2 mo post partum ($P = 0.60$; Figure 12). Concentrations of glucose in plasma were not effected by the hour of sampling after 14 d consuming predicted (NRC) MR diets ($P = 0.97$; data not shown), at maintenance ($P = 0.13$) or at 2 mo post partum ($P = 0.78$; Table 8).

Concentrations of insulin in plasma were not effected ($P > 0.57$) by MR x hour at any time in yr 2. Plasma concentrations of insulin did not differ among LMR, MMR and HMR cows at maintenance ($P = 0.74$) or at 2 mo post partum ($P = 0.90$; Figure 12). Plasma concentrations of insulin were not influenced (P = 0.25) by the hour of sampling at maintenance (Table 8). However, concentrations of insulin in plasma were greater ($P \leq 0.003$) at 0730 and 1100 h compared with 1430 h at 2 mo post partum (Table 8).

Maintenance energy requirements were not correlated with BW, BCS of the cows, or plasma concentrations of IGF-I, T_4, glucose, and insulin at maintenance (Table 9). Similarly, MR were not correlated with BW, BCS, or plasma concentrations of glucose or insulin at 2 mo post partum (Table 10). However, MR were negatively correlated with IGF-I (r = -0.38; $P = 0.05$) and tended to be positively correlated with T_4 (r = 0.31; $P = 0.12$) at 2 mo post partum. Body condition score was positively correlated with plasma concentrations of glucose (r = 0.45, $P = 0.02$) at maintenance, and with plasma concentrations of T_4 (r = 0.41; $P = 0.04$) at 2 mo post partum. Concentrations of IGF-I in

plasma were positively correlated with plasma concentrations of T_4 ($r = 0.53$, $P < 0.01$) at maintenance.

The adjusted coefficient of determination for a multiple linear regression model for MR was small. The full model included BW, BCS, IGF-I, T_4, glucose, and insulin. After the forward stepwise procedure the variables left in the model were IGF-I, T_4 and insulin. However, the adjusted R^2 (0.23) was very small. The final model was:

MR ($Kcal \cdot BW^{-0.75} \cdot d^{-1}$) = 0.07607 – 0.0001246 IGF-I + 0.02119 insulin + 0.00044864 T_4

Ruminal temperature from 3 to 7 d before parturition was not influenced ($P = 0.56$) by MR in LMR (39.05 ± 0.09 °C), MMR (38.94 ± 0.07 °C) and HMR (38.93 ± 0.07 °C) cows.

Proteins were separated over a pH range of 4 to 7, and size between 71 and 14 kDa (Figure 13), were analyzed. Abundant proteins (Figure 13) such as serum albumin (No 1), actin (No 25), tropomyosin (No 23 and 24), or myosin (No 62) were difficult to focus under a diversity of conditions while developing the procedures. From a total of 103 isolated protein spots, 78 proteins corresponding to 52 gene products were identified. Proteins were related to metabolism (33%), contractile apparatus (19%), cell structure (10%), cell defense (13%), and other processes (25%; Table 11; annotated in Figure 13). The largest protein identified was the 71 kDa heat shock cognate, and the smallest was the 14 kDa phosphohistidine phosphatase. Several proteins (16 of 78) were represented on more than one spot indicating the presence of several isoforms of the protein. The percentage of proteins matching bovine proteins in the databases was 95 %, and the rest were matched to human proteins. In most cases theorethical and experimental p*I* and

MW were similar. Nevertheless, differences in theoretical and experimental p*I* and MW of the proteins were present. The biggest difference between theoretical and experimental p*I* occurred with cofilin-2 (7.88 vs. 5.9). The biggest difference between theoretical and experimental MW was with creatinine kinase M-type (43.04 vs. 22.5 kDa), and pyruvate kinase isozime (58.0 vs 35.0 kDa). Proteins such as heat shock beta 1 and troponin T slow skeletal muscle were represented by 4 spots that differed mainly in their p*I*. Heat shock beta 1 was represented by spots 69, 71, 73, and 107 (Figure 13) and experimental p*I* were 5.69, 5.89, 6.24, and 6.6, respectively.

Protein abundance was evaluated using 8 gels and 24 images, because technical difficulties required the exclusion of 4 gels. Only proteins that were present in 21 or more of the 24 images were considered for analysis. Protein abundance tended ($P = 0.11$; Figure 14) to be greater in HMR cows for cofilin-2 (No 87 in Figure 13), and for the combination of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and/or troponin T fast skeletal muscle ($P = 0.16$; Figure 14; No 39 in Figure 13). The system did not separate GAPDH and troponin T fast skeletal muscle, and the 3-D images shows only one peak (Figure 15).

DISCUSSION

Differences in ambient temperature between years and different groups of cows may be responsible for the variations in MR ($Kcal \cdot BW^{-0.75} \cdot d^{-1}$) that averaged 89.2, 93.0 and 90.4 for yr 1, 2 and 3, respectively. Five cows were studied in both yr 1 and 2, and 4 of the 5 ranked in the same position relative to each other in the second yr. Other studies determined that the daily ME_m for mature nonlactating, nonpregnant Angus x Hereford cows was between 127 to 151 $Kcal \cdot BW^{-0.75} \cdot d^{-1}$ (Thompson et al., 1983; Ferrell and

Jenkins, 1985; and Reid et al., 1991), and was between 91.4 to 156.7 Kcal·$BW^{-0.75}$·d^{-1} for Angus cows (Ferrell and Jenkins, 1985; Solis et al., 1988; DiConstanzo et al., 1990; and Laurenz et al., 1991). Estimation of MR in this study was determined using the NE_m basis. Metabolizable energy for maintenance includes the heat increment of the feed. Deduction of the heat increment of feed from ME_m gives the NE_m (McDonald et al., 2002). Differences in physiological and environmental conditions, method to estimate MR, and other factors may account for variations in estimated MR among studies.

The greatest difference in MR between the most efficient cow and the least efficient cow was 29% and 24% for yr 1 and 2, respectively. This is consistent with other studies in which ME_m varied by 27 % in Angus cows (DiConstanzo et al., 1990), and 23% in Hereford steers (Derno et al., 2005). The coefficients of variation for MR in our study were 7% and 5% in yr 1 and 2, respectively. In Angus cows the CV for MR was 11% (DiConstanzo et al., 1990). These results corroborate the variation that exists in MR and the presence of more efficient cows within the same herd. Differences in MR between cows may be relatively expressed along different physiological states (Ferrell and Jenkins, 1985a; Montaño-Bermudez et al., 1990) and seasons (Laurenz et al., 1991). Maintenance energy requirements were 12% greater in Red Poll x Angus and Milking Shorthorn x Angus cows compared with Hereford x Angus cows during gestation and lactation (Montaño-Bermudez et al., 1990). Maintenance energy requirements were greater in Simmental compared with Angus cows during different seasons (Laurenz et al., 1991). Selection of mice for greater or lesser heat loss resulted in significant differences in heat loss after 15 generations (Nielsen et al., 1997a). Heritability for heat loss in mice is between 0.25 and 0.3 (Nielsen et al., 1997a). Maintenance energy requirements have a

moderate heritability in beef cattle (h^2 = 0.52; Hotovy et al., 1991). Selection of more efficient cows should be feasible.

Body condition score of the cows was not effected by MR after the trial. This indicates that when cows have similar BCS, cows with lower MR may be more efficient in the use of energy. Body condition score of cows was positively correlated with concentrations of glucose in plasma at maintenance. Similarly, loss of BCS was associated with reduced concentrations of glucose in feed restricted beef cows (Richards et al., 1989) and other ruminants (Trenkle, 1978). In addition, BCS was positively correlated with concentrations of insulin in plasma of primiparous beef cows (Lalman et al., 2000) and pregnant beef cows (Lents et al., 2005), and changes in plasma insulin usually correspond to similar changes in plasma concentrations of glucose (Richards et al., 1989). Body condition score of the cows was positively correlated with concentration of T_4 in plasma 2 mo post partum. In agreement, postpartum lactating beef cows with greater BCS had greater concentrations of T_4 in plasma compared with thinner cows on d 46, 60 and 67 post partum (Flores et al., 2008), and BCS accounted for 7% of the variation in concentrations of T_4 in plasma of pregnant beef cows (Lents et al., 2005).

Birth and weaning weights of calves were not influenced by MR of the dam. Milk production and weaning weights of calves are positively associated (Neville, 1962; Rutledge et al., 1971), indicating the MR did not influence milk production of cows in this study. Milk production potential was not associated with FI in primiparous lactating Hereford x Angus, Hereford x Simmental, and Hereford x Tarentaise cows (Freking and Marshall, 1992). Cows consuming less energy relative to their milk production were the most efficient (Freking and Marshall, 1992). Perhaps milk production was not associated

with MR and/or more efficient cows made better use of the energy available for milk production. Even though, milk production was positively associated with MR among different crossbreeds and types (Ferrell and Jenkins, 1984), only 23 % of the variation in MR was accounted for the differences in milk production (Montano-Bermudez and Nielsen, 1990). Variation in MR for cows in the current experiment was not associated with milk production since weaning weights of the calves were not influenced by MR of the cows.

Days to resumption of luteal activity after parturition was not influenced by MR of cows. Nutrition and BCS at parturition are major factors influencing reproductive performance in beef cows (Randel et al., 1990; Wettemann et al., 2003). Body condition score of cows at calving was not influenced by MR. Low MR cows may be more efficient in the use of energy. Selection of mice for low or high heat loss, and hence MR, influenced reproductive performance. Mice with reduced MR had decreased ovulation rate and litter size compared with high MR mice (Nielsen et al., 1997b). In contrast, MR did not influence litter size in mice exposed to hot or cold environments. Litter size in mice was influenced by MR under normal temperature conditions, and medium MR mice had greater litter size compared with the low or high MR mouse lines (Kgwatalala et al., 2004). In our study, weaning weights of calves were not influenced by MR of cows, which makes LMR cows more efficient by decreasing the inputs/outputs ratio. Evaluation of the effect of selection for LMR of cows on reproductive performance of the cows will require further investigation.

Concentrations of IGF-I in plasma were negatively correlated with MR when cows were grazing ad libitum at 2 mo post partum. Insulin-like growth factor I is

primarily produced by the liver in response to growth hormone (GH; Jones and Clemmons, 1995; Keisler and Lucy, 1996). Cattle with a negative energy balance usually have increased concentrations of GH in plasma and decreased IGF-I (Richards et al., 1991; Keisler and Lucy, 1996; Bossis et al., 1999). When cows are in negative energy balance, receptors for GH (GHR) in the liver are downregulated, therefore the normal stimulatory action of GH on the synthesis of IGF-I becomes uncoupled and IGF-I secretion by the liver is reduced despite high plasma concentrations of GH (Thissen et al., 1994). Realimentation reverses the uncopling of the GH-IGF-I system (Thissen et al., 1994). Concentrations of hepatic GHR and plasma IGF-I are positively related to nutrient uptake (Donaghy and Baxter, 1996). The negative correlation observed between MR and IGF-I while cows were grazing ad libitum could be the consequence of down regulation of hepatic GH receptors, uncoupling of the GH-IGF-I axis, and decreasing IGF-I secretion. Under similar conditions, even when cows had similar energy reserves, nutrients available may have been inadequate for cows with greater MR to stimulate IGF-I secretion by the liver, whereas cows with lower MR, required less energy to stimulate IGF-I secretion.

Breed influences concentrations of IGF-I in serum during the post partum period (Spicer et al., 2002). Concentrations of IGF-I in serum increased in grazing Brahman cows, Brahman x Angus, and Brahman x Charolais cows during wk 2 and 7 post partum, while concentrations of IGF-I in serum of Angus, Charolais or Angus x Charolais cows were constant (Spicer et al., 2002). The differences between species may be related to differences in MR. *Bos indicus* require 10% less energy than *B. taurus* and crosses are intermediate in growing cattle (NRC, 1996). Brahman x Hereford cows had lesser MR

compared with Red Poll cows (Reid et al., 1991) and Nellore cows had lesser MR compared with crosses of Nellore with *B. taurus* (Calegare et al., 2007). Brahman and Brahman influenced cows had increased concentrations of IGF-I in serum, and these cows may also have lesser MR, compared with Angus, Charolais or Angus x Charolais cows. Cows with different MR may have different regulation of IGF-I secretion, and cows with lesser MR require less energy to stimulate secretion of IGF-I.

Cows were fed at 0830 h and concentrations of IGF-I in plasma were greater at 0730 and 1100 h compared with those at 1430 h. In agreement, pregnant beef cows had greater concentrations of IGF-I in plasma after restricted from feed and water for 18 h compared with samples taken 1 h after feeding (Lents et al., 2005). Plasma concentrations of IGF-I decreased after fasting in rats (Maes et al., 1983), pigs (Dauncey et al., 1990) and heifers (Spicer et al., 1992), and increased 6 to 9 h after feeding in lactating dairy cows at 2 mo post partum (Wylie et al., 2008). Growth hormone tended to decrease after feeding in dairy cows (Sutton et al., 1988), which may be a consequence of increased IGF-I in plasma and the negative feedback of IGF-I on GH release. Feeding did not influence plasma concentrations of IGF-I in dairy cows 1, 3 or 7 mo post partum (Wylie et al., 2008) or in dairy calves (Vicari et al., 2008). Differences among species, differences in the physiological state of animals, diets, and feeding times may be responsible for the different effects of feeding on plasma concentrations of IGF-I.

Concentrations of T_4 in plasma tended to be greater in HMR cows compared with LMR and MMR cows when cows were fed the maintenance diet. Concentrations of T_4 in plasma are associated with FI (Richards et al., 1995; Ciccioli et al., 2003). Feed restriction resulted in decreased plasma concentrations of T_4 and realimentation increased

concentrations of T_4 in plasma of nonlactating beef cows (Richards et al., 1995). Greater energy intake of HMR cows per $BW^{-0.75}$ compared with the LMR cows could be related to greater plasma concentrations of T_4 in HMR, and the positive correlation between T_4 and IGF-I supports this suggestion. However, plasma concentrations of T_4 were not influenced by MR when cows grazed ad libitum. Similarly, serum concentrations of T_4 were not influenced by MR of mice when fed ad libitum (Kgwatalala et al., 2004). A positive correlation between T_4 and BCS in this study agrees with a tendency for lesser concentrations of T_4 in thin compared with fat beef cows (Rasby et al., 1991).

Diurnal variation in T_4 in this study was also observed in beef cows (Lamoglia et al., 1997) and dairy heifers (Bitman et al., 1984). Plasma concentrations of T_4 tended ($P < 0.08$) to be less at 0300 h, intermediate at 1100 h and greatest at 1900 h in pregnant beef cows fed a total mixed ration (Lamoglia et al., 1997). Plasma concentrations of T_4 in pregnant dairy heifers were less during the morning and greater during the afternoon and the increase in T_4 occurred shortly after feeding (Bitman et al., 1984). Collectively, these results indicate that the diurnal variation in T_4 is related to variation in nutrient availability in plasma.

Maintenance energy requirements did not influence concentrations of glucose and insulin in plasma. Greater concentrations of insulin in plasma at 0730 h and 1100 h compared with concentrations of insulin at 1430 h occurred when cattle grazed pasture but concentrations of insulin in plasma were similar at the three sampling times when cows were fed a maintenance diet once daily. Changes in plasma insulin usually correspond to changes in plasma concentrations of glucose (Richards et al., 1989). Concentrations of insulin in plasma increased within 1 h after feeding beef cows (Lake et

al., 2006), and 3 to 5 h after feeding dairy cows (Sutton et al., 1988; Aleman et al., 2007; Wylie et al., 2008). The increase in insulin after feeding was evident when dairy cows were fed twice a day but not when cows were fed 6 times a day and concentrations of glucose in plasma were constant (Sutton et al., 1988). Pregnant beef cows had greater concentrations of insulin in plasma after restricted from feed and water for 18 h compared with samples from fed cows (Lents et al., 2005). Diets composition and feeding management may influence plasma concentrations of glucose and secretion of insulin.

Multiple regression analysis indicated that IGF-I, T_4 and insulin were not adequate to predicted MR of cows. The coefficient of determination of the model was small. Milk production accounted for 23% of the variation in MR of beef cattle (Montano-Bermudez et al., 1990). This indicates that variation in MR may be explained by factors other than IGF-I, T_4, insulin, or milk production.

Ruminal temperatures did not differ for cows with different MR. Rectal temperature was positively associated with MR of beef steers ($r = 0.70$, $P = 0.06$; Derno et al., 2005) and mice (Kgwatalala et al., 2004). Variations in ruminal temperature as a consequence of fermentation (Dye, 2005; Reynolds et al., 1991) and water consumption (Brod et al., 1982; Dye, 2005) could obscure variations in ruminal temperature associated with other physiological process.

Proteins from *Longissimus dorsi* of beef cows with different MR were separated and identified for the first time. Similar to our results, 25% of the proteins identified from *Semitendinosus* muscle of beef steers were related to metabolism (26%), contractile apparatus (15%), cell structure (17%), cell defense (16%), and other processes (26%; Bouley et al, 2004). In the current study, 21% of the proteins were represented by more

than 1 spot, similarly in bovine *Semitendinosus* muscle 25% of the proteins were represented by more than one spot (Bouley et al., 2004).

Differences in theoretical and experimental p*I* and MW of proteins could be attributed to co- or post-translational modifications (PTM) such as glycosilation, phosphorylation and ptoteolytic cleavage. Cofilin-2 had the greatest difference in experimental p*I* compared with theoretical p*I*, and may be the consequence of post-translational phosphorylations (Akira Yamagata, 2002). In agreement, Cofilin-2 had the greatest difference in experimental p*I* compared with theoretical p*I*, in beef steers *Semitendinosus* muscle (Bouley et al., 2004), bull calves *Longissimus dorsi* and *Semitendinosus* muscle (Jia et al., 2006b) postmortem, and human *Vastus lateralis* muscle (Gelfi et al., 2003). Difference between theoretical and experimental MW observed for creatinine kinase M-type could be attributed to glycosylation (Spiro et al., 1992). Some proteins were represented by different spots, and the presence of different spots for the same protein suggests PTM, and/or the presence of several isoforms of the same protein. Heat shock beta 1 was represented by 4 spots that differ basically in their p*I*. This may be a consequence of PTM and change in their p*I* as previously observed for Hsp 70 (Gutierrez and Guerriero, 1995).

Cofilin-2 tended to be more abundant in HMR cows compared with LMR cows. Cofilin is an actin-regulatory protein required for reorganization of actin filaments, and recycles older ADP-F-actin to maintain ATP-G-actin pool for sustained motility in vivo (Bamburg, 1999). Cofilin is associated with contractile and immune responses of eukaryotes (Bamburg, 1999). In humans, cofilin is critical for activation of the immune system (Samstag et al., 2003). In bovine, cofilin has been associated with the immune

system response to stressors. Cofilin was increased in the respiratory tract of Holstein calves after dexamethasone treatment, indicating a stressor-dependent increase in this protein (Mitchell et al., 2007). Moreover, reduced experimental p*I* of cofilin in the present study could be due to activation of cofilin by dephosphorylation (Rosenblatt et al., 1997). Increased contractile and/or immune responses, suggested by the greater abundance of cofilin-2, may be responsible, in part, for the greater energy expenditure in HMR cows compared with the LMR cows.

Glyceraldehyde-3-phosphate dehydrogenase and troponin T fast skeletal muscle (TnT) were 2 proteins identified in the same spot, and this is possible since they have similar MW. Nevertheless the theoretical p*I* of GAPDH is 8.52 and the experimental p*I* was 6.56 in this study; this could be the consequence of PTM (Akira Yamagata, 2002). The tendency for a greater abundance of GAPDH in HMR cows in our study is consistent with previous results in dairy cows. Glyceraldehyde-3-phosphate dehydrogenase was more abundant in hypothalamic tissue from ad libitum fed compared with energy restricted cows (Kuhla et al., 2007). Glyceraldehyde-3-phosphate dehydrogenase participates in glycolysis, indicating that greater substrate is being used as an energy source to maintain energy homeostasis in HMR cows that consumed more daily energy per unit of metabolic BW compared with LMR cows. The protein TnT was identified in the same spot as GAPDH. Troponin T fast skeletal muscle is a subunit of troponin that attaches troponin and tropomyosin, helps tropomyosin to position on actin, and modulates muscle contraction (Stefancsik et al., 2003; Murakami et al., 2008). The fact that other spot was also identified as TnT, with no differential expression observed, leaves the doubt of which of these two proteins caused the tendency. This is the first

report that identifies proteins from bovine *Longissimus dorsi*. Our data and the developed procedure may be useful in further proteomic analyses in energy efficiency, meat quality and other studies involving bovine muscle.

The current study corroborates the within herd variation in MR and the opportunity that this represents for beef production especially because differences in cow and calves performance were not detected. Individually, T_4, glucose and insulin may not be biomarkers of MR but possibly IGF-I either alone or combined with other hormones, could predict MR, use of concentrations of hormones in plasma as biomarkers for MR will require further investigation. Cofilin-2 is a potential biomarker for MR.

Residual feed intake (RFI) studies found that steers with FI less than predicted amounts (low RFI) perform similar to steers with FI greater than predicted amounts or high RFI (Arthur et al., 2001). Residual feed intake is negatively correlated with ME availability in beef steers (Nkrumah et al., 2006), and positively correlated with heat production (Basarab et al., 2003; Nkrumah et al., 2006). The greater metabolizability of feed in low RFI steers, compared with high RFI steers, might be attributed, at least in part, to the decreased methane production (Nkrumah et al., 2006; Hegarty et al., 2007) and increased apparent digestibility in steers with low RFI (Nkrumah et al., 2006). The lesser heat production in low RFI steers compared with high RFI steers may be the consequence of variations in metabolic efficiency (Nkrumah et al., 2006). Steers with lesser RFI consumed less DMI (Basarab et al., 2003; Nkrumah et al., 2006) and this was positively associated with visceral organ mass (Basarab et al., 2003). Greater MR is associated with greater visceral organ mass in pigs (Tess et al., 1984; Noblet et al., 1999), sheep (Burrin et al., 1990), and cattle (Ferrell and Jenkins, 1984; Wagner et al., 1988;

DiCostanzo et al., 1990; Reynolds et al., 1991; Freetly et al., 2006). Low RFI steers produce less heat, therefore have lower MR, and have decreased size of visceral organ mass compared with high RFI steers. The biological basis for efficient animals, steers with low RFI or cows with low MR, may be similar. Methane production and digestibility of dietary DM and CP in beef cows with different MR should be evaluated.

IMPLICATIONS

Variation in maintenance energy requirement of nonlactating, pregnant, Angus x Hereford cows ranged from 24% to 29%. This offers the opportunity to improve efficiency of beef production by selecting more efficient cows. Cows that required less energy for maintenance weaned calves of similar weight compared with cows that had greater maintenance energy requirements. Variation in maintenance energy requirement of beef cows could be associated with plasma concentrations of IGF-I under grazing conditions. Concentrations of T_4, glucose and insulin in plasma, as well as ruminal temperature, may not work individually as a biomarker for maintenance energy requirement. Proteomic analyses of bovine muscle identified that cofilin-2 tended to be more abundant in cows with high maintenance energy requirement and may have application as a biomarker for maintenance energy requirement. Identification of biomarkers for maintenance energy requirement will allow identification and selection of more efficient cows and therefore improve efficiency of production. Cows that consume less feed and produce calves with similar weaning weights will enhance the sustainability of the environment.

Table 4. Labeling reactions and IPG strip rehydration arrangement of protein extractions from *Longissimus dorsi* of nonlactating pregnant beef cows with low or high maintenance energy requirements (MR) during the period of constant BW in yr 2 and yr 3[1]

	Fluorescent dyes for labeling used[2]			Sample	
Sample:	Cy 3	Cy 5	Cy 2	Amount, µg	IPG strip
High MR	*			50	1
Low MR		*		50	1
Low MR	*			50	2
High MR		*		50	2
High MR	*			50	3
Low MR		*		50	3
Low MR	*			50	4
High MR		*		50	4
Low MR	*			50	5
High MR		*		50	5
High MR	*			50	6
Low MR		*		50	6
Internal pooled standard[3]			*	50	All

[1] Maintenance energy requirement of a cow was classified as low (> 0.5 SD less than mean) or high (> 0.5 SD more than mean).

[2] Protein extractions were labeled with fluorescent dyes (CyDyes™, GE Healthcare Bio Sciences, Amersham, Piscataway, NJ, 50 µg of protein/400 pmol of dye), during 30 min at 4 °C, in the dark.

[3] Internal pooled standard was prepared by pooling equal aliquots of all protein extractions for cows with low and high MR and was included on all gels (50 µg·gel^{-1}).

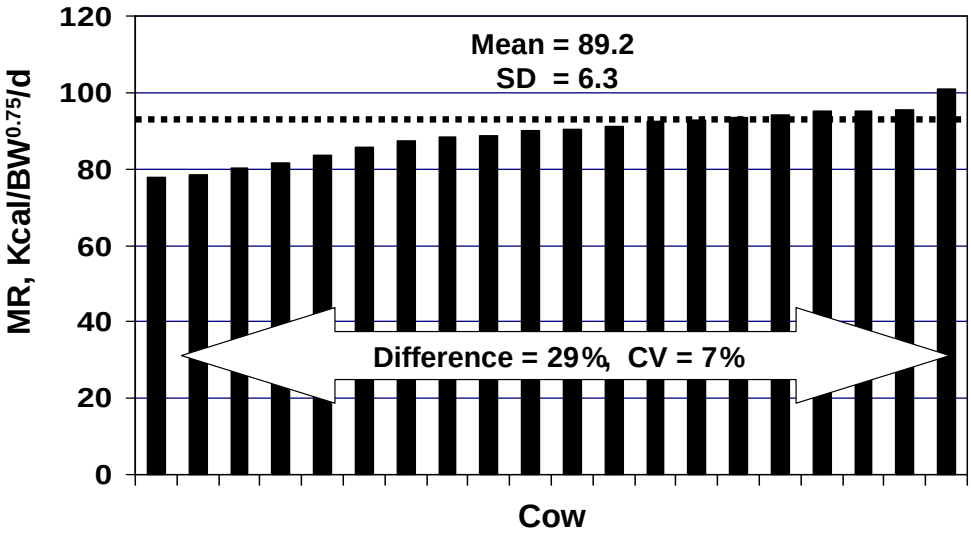

Figure 4. Maintenance energy requirement (MR, Kcal·$BW^{-0.75}$·d^{-1}) of nonlactating, pregnant beef cows, and mean estimated MR (93.9, NRC 1996, Level 1 Model) in yr 1. Bars represent actual MR of each cows (n = 20). Dotted line represents mean estimated MR (NRC,1996). Difference is the percentage of the difference in MR for the cow with the greatest MR and the cow with the least MR.

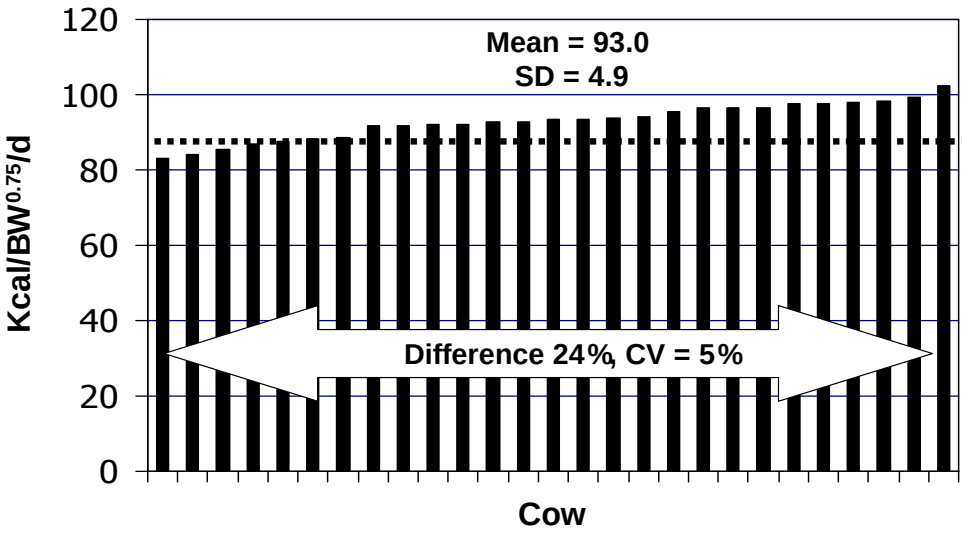

Figure 5. Maintenance energy requirement (MR, Kcal·$BW^{-0.75}$·d^{-1}) of nonlactating, pregnant beef cows, and mean estimated MR (87.3, NRC 1996, Level 1 Model) in yr 2. Bars represent actual MR of each cows (n = 27). Dotted line represents mean estimated MR (NRC,1996). Difference is the percentage of the difference in MR for the cow with the greatest MR and the cow with the least MR.

Table 5. Least squares mean maintenance energy requirements (MR) of nonlactating pregnant beef cows with low (L), medium (M), or high (H) MR during the period of constant BW in yr 1 (21 d) and yr 2 (28 d) [1]

	MR group[2]			
Item	L	M	H	SEM
Yr 1				
Cows, n	6	6	8	
MR	81.17[a]	89.33[b]	95.00[c]	1
Yr 2				
Cows, n	7	10	10	
MR	86.30[a]	92.81[b]	97.88[c]	0.56

[1] Maintenance energy requirements (MR, NE_m, $Kcal \cdot BW^{-0.75} \cdot d^{-1}$) is presented as least squares mean per group.

[2] Group were defined as low (> 0.5 SD less than mean, L), medium (± 0.5 SD of mean, M) or high (> 0.5 SD more than mean, H).

[a,b,c] Means within a row with a different superscript differ ($P < 0.05$).

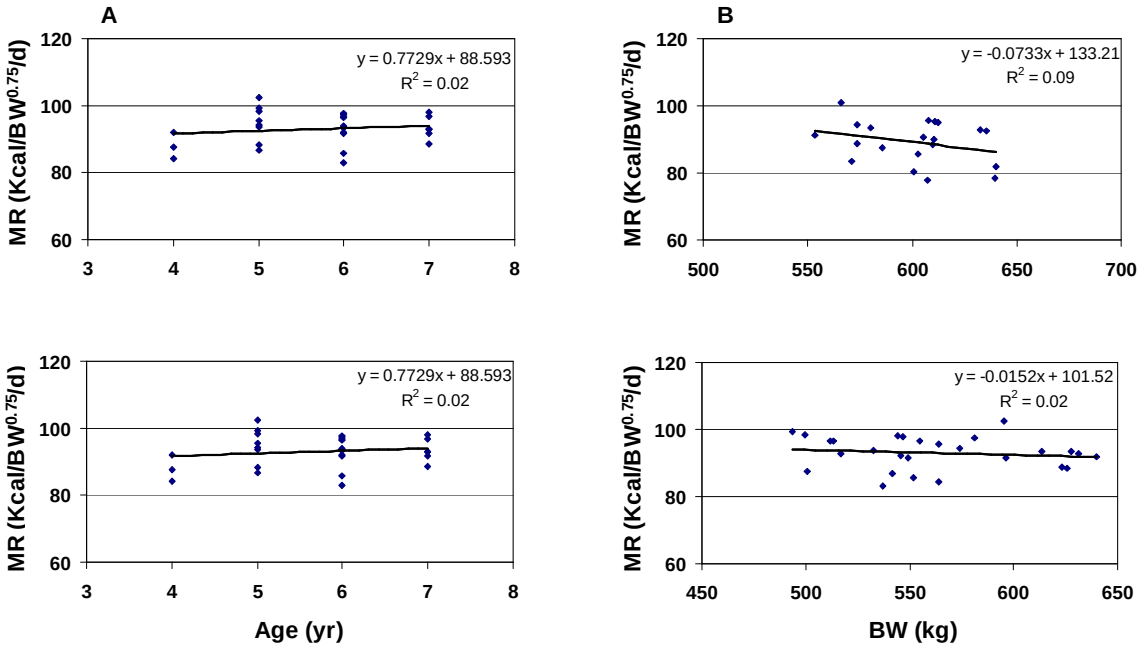

Figure 6. Least squares regressions for maintenance energy requirements (MR) of nonlactating pregnant beef cows with age (Panel A) or constant BW (panel B) during 21 d in yr 1 (n = 20) and 28 d in yr 2 (n = 27).

Table 6. Body weight changes and BCS of beef cows with low (L), medium (M), or high (H) maintenance energy requirements in yr 1 and yr 2[1]

	MR group[2]				
Item	L	M	H	SEM	*P* value
Year 1					
Cows, n	6	6	8		
BW at maintenance	610	590	602	10	0.38
BCS at maintenance	5.2	5.1	5.0	0.2	0.43
BCS at parturition	5.2	5.1	5.1	0.1	0.73
BW change at two mo post partum, kg	-72.7	-58.2	-86.0	-8.7	0.11
BW change at weaning, kg	-13.1	-1.7	-21.2	-11.4	0.50
BCS at weaning	5.1	4.9	4.6	0.2	0.08
Year 2					
Cows, n	7	10	10		
BW at maintenance	563	583	541	14	0.10
BCS at maintenance	5.1	5.3	5.1	0.2	0.08
BW change one mo. after trial, kg	+36.7[a]	+53.1[b]	+59.9[b]	+5.0	0.02
BCS at parturition	4.8	5.1	4.9	0.1	0.17
BW change at two mo. postpartum, kg	- 23.6	-17.9	-12.5	-6.3	0.50
BCS two mo. post partum	4.5	4.8	4.6	0.1	0.30
BW change at weaning, kg	+5.8	+8.8	+9.8	+6.7	0.92
BCS at weaning	4.7	4.8	4.5	0.1	0.27

[1] Body weight at maintenance is the mean body weight during the period of constant BW. Body weight change was the difference with the BW at the end of MR determination at 8 mo of gestation (yr 1) or 7 mo of gestation (yr 2).

[2] Group were defined as low (> 0.5 SD less than mean, L), medium (± 0.5 SD of mean, M) or high (> 0.5 SD more than mean, H).

[a,b] Means within a row with a different superscript differ ($P < 0.05$).

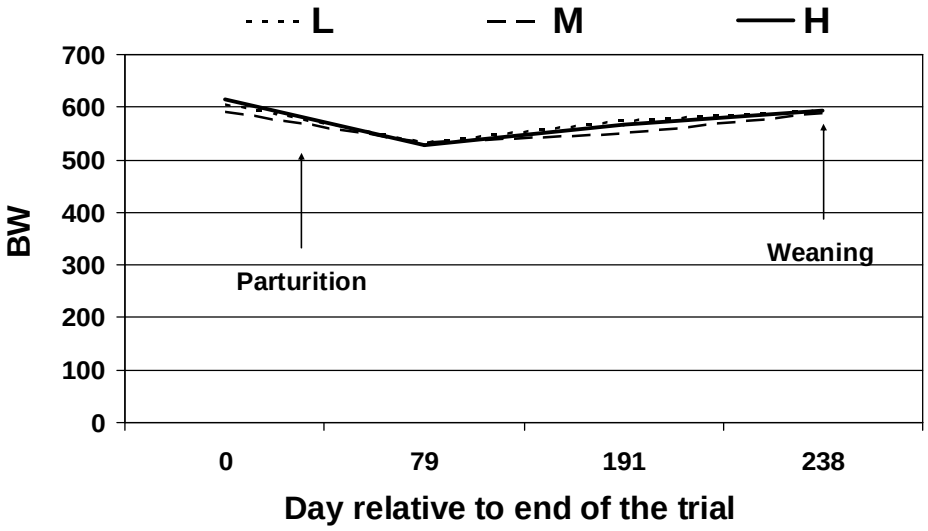

Figure 7. Body weight (BW) of beef cows with low (L, n = 6), medium (M, n = 6) or high (H, n = 8) maintenance energy requirement in yr 1. Groups were defined as low (> 0.5 SD less than mean), medium (± 0.5 SD of mean) or high (> 0.5 SD more than mean). Average SE across days was 10 kg.

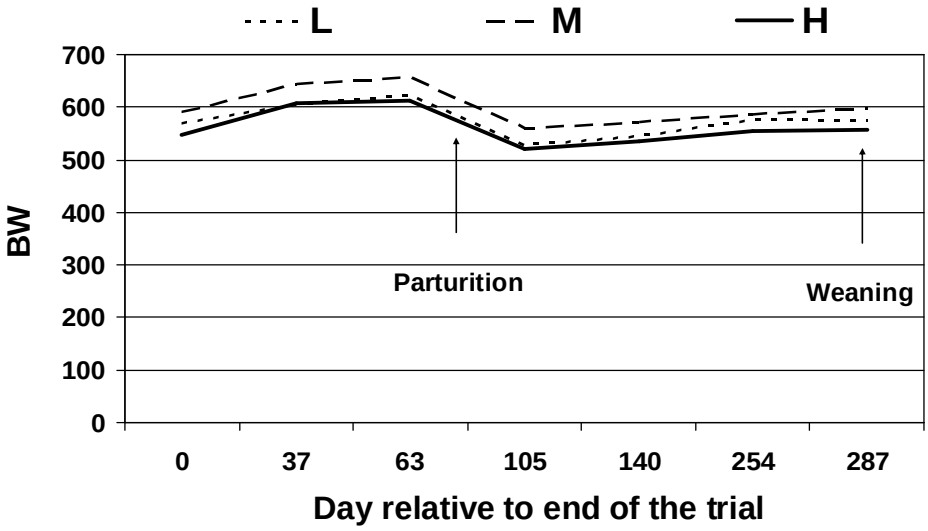

Figure 8. Body weight (BW) of beef cows with low (L, n = 7), medium (M, n = 10) or high (H, n = 10) maintenance energy requirement in yr 2. Groups were defined as low (> 0.5 SD less than mean), medium (± 0.5 SD of mean) or high (> 0.5 SD more than mean). Average SE across days was 16 kg.

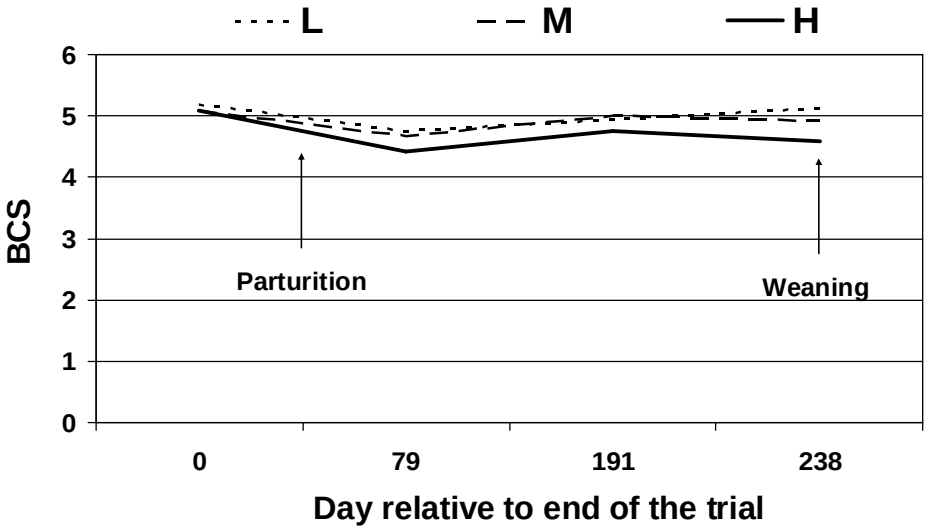

Figure 9. Body condition score (BCS) of beef cows with low (L, n = 6), medium (M, n = 6) or high (H, n = 8) maintenance energy requirements in yr 1. Groups were defined as low (> 0.5 SD less than mean), medium (± 0.5 SD of mean) or high (> 0.5 SD more than mean). Average SE across days was 0.1.

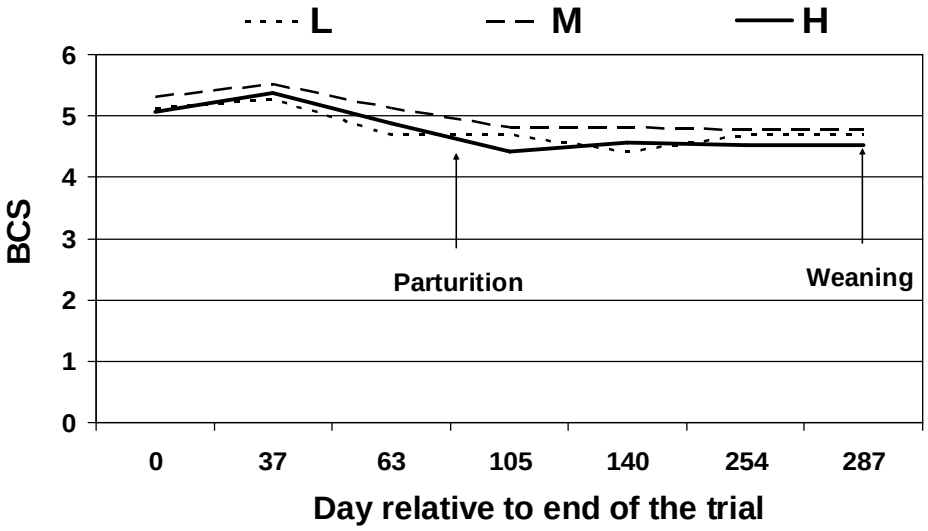

Figure 10. Body condition score (BCS) of beef cows with low (L, n = 7), medium (M, n = 10) or high (H, n = 10) maintenance energy requirements in yr 2. Groups were defined as low (> 0.5 SD less than mean), medium (± 0.5 SD of mean) or high (> 0.5 SD more than mean). Average SE across days was 0.1.

Table 7. Body weight of calves and luteal activity of beef cows with low (L), medium (M), or high (H) maintenance energy requirements in yr 1 and yr 2

	MR group[1]				
Item	L	M	H	SEM	*P* value
Year 1					
Calves, Cows, n	6	6	8	-	-
Calves birth BW, kg	38	40	40	3.5	0.59
Calves adjusted 205 d BW, kg	208	195	197	8.5	0.60
Year 2					
Calves, Cows, n	6	10	9	-	-
Calves birth BW, kg	41	42	42	1.3	0.72
Calves adjusted 205 d BW, kg	259	252	254	4.4	0.61
Initiation of luteal activity, d	49	45	50	3.6	0.57
Both Years					
Calves birth BW, kg	39	42	41	2	0.20
Calves adjusted 205 d BW, kg	234	221	226	27	0.23

[1] Group were defined as low (> 0.5 SD less than mean, L), medium (± 0.5 SD of mean, M) or high (> 0.5 SD more than mean, H).

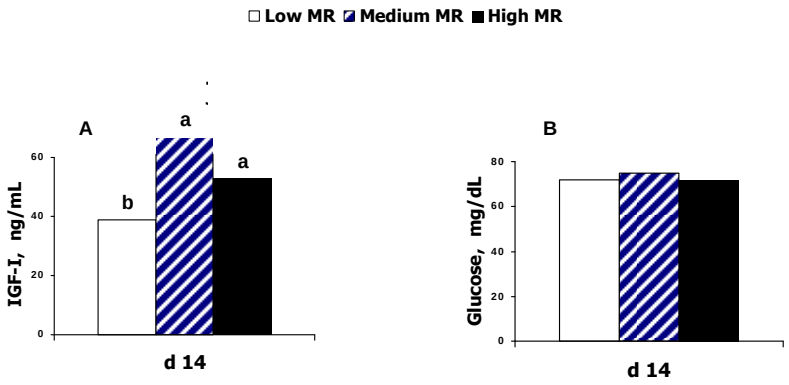

Figure 11. Least squares mean concentrations of IGF-I (A) and glucose (B) for Low (n = 7), Medium (n = 10) and High (n = 10) maintenance energy requirement (MR) cows on d 14 of the trial, after cows consumed the estimated maintenance level (Level 1 Model; NRC, 1996) for 14 d in yr 2. Groups were defined as Low (> 0.5 SD less than mean), Medium (± 0.5 SD of mean) or High (> 0.5 SD more than mean). Standard errors averaged across treatments were 5.1 and 1.9 for IGF-I and glucose, respectively. [a,b] Means without common letter differ ($P < 0.05$).

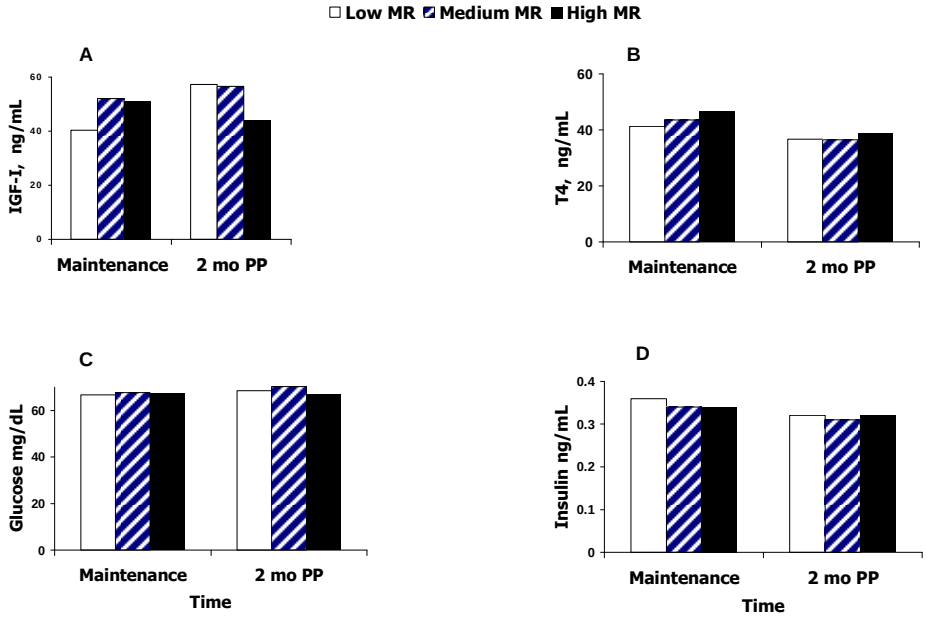

Figure 12. Least squares mean concentrations of IGF-I (A), T_4 (B), glucose (C), and insulin (D), in plasma for Low (n = 7), Medium (n = 10) and High (n = 10) maintenance energy requirement (MR) cows at maintenance and at 2 mo post partum (PP) in yr 2. Groups were defined as Low (> 0.5 SD less than mean), Medium (± 0.5 SD of mean) or High (> 0.5 SD more than mean). Standard errors averaged across treatments were 4.8, 2, 1.6, and 0.02, for IGF-I, T4, glucose, and insulin, respectively.

Table 8. Hour effect on concentrations of IGF-I, T_4, glucose, and insulin in plasma of beef cows fed to maintenance or grazing in yr 2[1]

	hour				
Item	0730	1100	1430	SEM	*P* value
Fed to maintenance					
IGF-I, ng/mL	49.3 [a] (27) [2]	48.3 [a] (27)	45.9 [b] (27)	2.6	0.04
T_4, ng/mL	41.1 [a] (25)	44.4 [b] (25)	45.9 [b] (25)	1.7	0.06
Glucose, mg/dL	68.5 (27)	65.2 (27)	67.9 (27)	1.8	0.16
Insulin, ng/mL	0.35 (26)	0.36 (23)	0.32 (24)	0.02	0.25
At 2 mo post partum[2]					
IGF-I, ng/mL	55.7 [a] (25)	55.9 [a] (24)	46.5 [b] (22)	3.3	0.007
T_4, ng/mL	33.8 [a] (26)	39.6 [b] (24)	38.7 [b] (25)	1.0	< 0.001
Glucose, mg/dL	68.65 (27)	69.37 (27)	68.06 (27)	2.0	0.75
Insulin, ng/mL	0.35 [a] (22)	0.33 [a] (23)	0.27 [b] (25)	0.02	0.002

[1] Blood samples were taken from cows (nonlactating, pregnant cows) after fed to actual maintenance for 28 d or from cows (lactating, nonpregnant) when grazing native prairie grass pasture at 2 mo post partum. Samples were taken after cows were restricted from feed and water for 18 h at 0730 h, or after cows had access to feed and water at 1100 and 1430 h.

[2] Sample size in parenthesis.

[a,b,c] Means within a row with a different superscript differ ($P < 0.05$).

Table 9. Simple correlation coefficients (r/*P* value), among, maintenance energy requirement (MR, NEm, Kcal·$BW^{0.75}$·d^{-1}), BW, BCS, IGF-I, T_4, glucose, and insulin of nonlactating, nonpregnant beef cows (n = 27) at maintenance, after 28 d of constant BW, in yr 2[1]

	MR	BW	BCS	IGF-I	T4	Glucose	Insulin
MR		-0.13	-0.14	0.27	0.28	-0.11	-0.20
		0.51	0.47	0.18	0.16	0.59	0.33
BW			0.12	-0.11	-0.27	0.06	0.20
			0.56	0.58	0.17	0.75	0.31
BCS				-0.15	0.14	0.45	-0.29
				0.46	0.47	0.02	0.15
IGF-I					0.53	0.08	0.31
					<0.01	0.68	0.12
T_4						0.11	0.25
						0.57	0.22
Glucose							0.10
							0.60

[1] Concentrations of hormones in plasma were a mean of three samples taken on the same day at 0730, 1100 and 1430 h.

Table 10. Simple correlation coefficients (r/*P* value), among, maintenance energy requirements (MR, NEm, Kcal·$BW^{-0.75}$·d^{-1}), BW, BCS, IGF-I, T_4, glucose, and insulin of nonlactating, nonpregnant beef cows (n = 26) at 2 mo post partum when cows were grazing, in yr 2[1]

	MR	BW	BCS	IGF-I	T_4	Glucose	Insulin
MR		0.04	0.11	-0.38	0.31	-0.17	0.10
		0.84	0.61	0.05	0.12	0.40	0.61
BW			0.21	0.22	0.10	0.22	0.32
			0.31	0.29	0.64	0.28	0.12
BCS				0.04	0.41	0.30	-0.02
				0.84	0.04	0.14	0.90
IGF-I					0.04	0.11	0.23
					0.83	0.57	0.25
T_4						0.04	-0.19
						0.83	0.34
Glucose							0.17
							0.40

[1] Concentrations of hormones in plasma were a mean of three samples taken on the same day at 0730, 1100 and 1430 h.

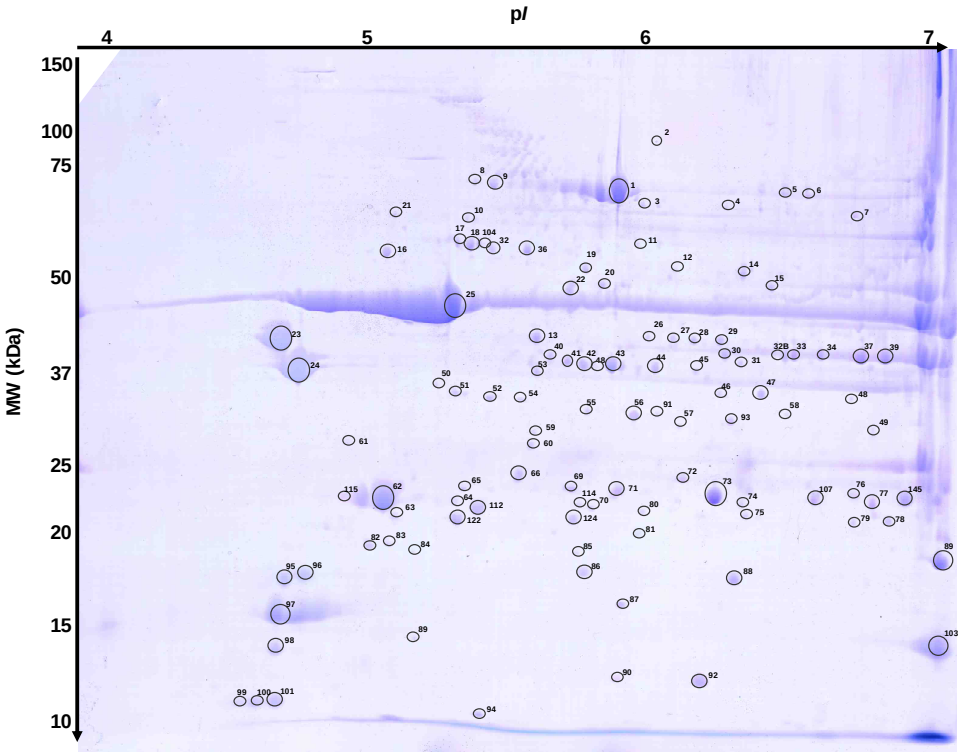

Figure 13. Bovine LM 2-DE Gel. Proteins were horizontally separated on an IPG gel strip (pH 4–7) and vertically on a 12.0% SDS PAGE gel (24 x 20 x 0.1 cm^3). Protein loaded was 470 µg, and gel was stained using commassie blue. Excised protein spots are circled and numbered in the gel.

Table 11. Protein identification and function from *Longissimus dorsi* biopsies of beef cows

Gel No	Name	SWISS-PROT/NCBI accession number	Orbitrap S. cov.	Orbitrap Prob.	MALDITOF M. score	Theort. MW (KDa) Orb.	Theort. MW (KDa) MALD.	Experimental MW (KDa)	Theoretical /Exp. pI
	Metabolism								
4	Phosphoglucomutase-1	PGM1_BOVIN/122132319	7%	100%		61.57		67.00	6.33/6.28
7	Pyruvate kinase isozyme M1	KPYM_HUMAN/194670470	9%	100%		58.00		64.00	7.95/6.75
11	Pyruvate dehydrogenase protein X component, mitochondrial	ODPX_HUMAN/12643417	8%	100%		54.11		58.00	5.39/5.98
14	Alpha-enolase	ENOA_BOVIN/87196501	9%	100%	86	47.31	47.30	52.00	6.37/6.33
16	ATP synthase subunit beta, mitochondrial	ATPB_BOVIN/114543	18%	100%		56.27		57.00	5.0/5.04
19	Cytochrome b-c1 complex subunit 1, mitochondrial	QCR1_BOVIN/10720406	11%	100%		52.72		53.00	5.46/5.77
30	Beta-enolase	ENOB_BOVIN/122140864			84		47.07	36.00	7.74/6.27
31	Aldose reductase	ALDR_BOVIN/162652□	9%	100%	51	35.90	33.95	35.30	5.76/6.32
39	Glyceraldehyde-3-phosphate dehydrogenase	G3P_BOVIN/85682743	8%	100%		35.85		35.60	8.52/6.56
45	Pyruvate kinase isozyme M1	KPYM_HUMAN/194670470	4%	100%	42	58.00	57.91	35.00	7.95/6.17
53	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	ODPB_BOVIN/116242689	10%	100%		39.11		34.80	5.39/5.59
70	Adenylate kinase isoenzyme 1	KAD1_BOVIN/109940090	10%	100%		21.65		22.50	8.40/5.79
76	Triosephosphate isomerase	TPIS_BOVIN/61888856	8%	100%		26.67		21.00	6.51/6.75
77	Creatine kinase M-type	KCRM_BOVIN/4838363	7%	100%	66	43.04	42.94	22.50	6.63/6.8
79	Adenylate kinase isoenzyme 1	KAD1_BOVIN/61888850□	20%	100%	81	21.65	21.65	21.00	8.40/6.75
81	ATP synthase subunit d, mitochondrial	ATP5H_BOVIN/114686	12%	100%		18.68		20.20	5.99/5.97
94	Phosphohistidine phosphatase 14 kDa	PHP14_BOVIN/115497372□	16%	100%	60	13.91	13.92	10.70	5.49/5.35
112	Apolipoprotein A-I precursor	APOA1_BOVIN/113988			70		30.26	22.20	5.36/5.36
114	Apolipoprotein A-I precursor	APOA1_BOVIN/113988			64		30.26	22.50	5.36/5.73
145	Triosephosphate isomerase	TPIS_BOVIN/61888856	20%	100%	75	26.67	26.67	22.80	6.51/6.92
32B	Beta-enolase	ENOB_BOVIN/122140864	8%	100%	82	47.00	47.07	36.20	7.74/6.48
	Cell defensive								
4	Stress-induced-phosphoprotein 1	STIP1_BOVIN/10720406	8%	100%		62.47		67.00	6.08/6.28
9	Heat shock cognate 71 kDa protein	HSP7C_BOVIN/157832109□	21%	100%	137	70.88	42.39	72.00	5.37/5.43
63	Peroxiredoxin-2	PRDX2_BOVIN/22095988	12%	100%		21.93		22.00	5.37/5.04
69	Heat shock protein beta-1	HSPB1_BOVIN/85542053			50		22.38	23.50	5.98/5.69
71	Heat shock protein beta-1	HSPB1_BOVIN/85542053	20%	100%	96	22.38	22.38	23.50	5.98/5.89
72	Peroxiredoxin-6	PRDX6_BOVIN/5902790	17%	100%	86	25.05	25.05	24.50	6.02/6.11
73	Heat shock protein beta-1	HSPB1_BOVIN/85542053			100		22.38	23.00	5.98/6.24
88	Heat shock protein beta-6	HSPB6_BOVIN/116248099	14%	100%	62	17.45	17.46	17.80	5.95/6.29
89	Alpha-crystallin B chain	CRYAB_BOVIN/117384	16%	100%	118	20.05	20.02	19.00	6.76/7.05
107	Heat shock protein beta-1	HSPB1_BOVIN/85542053			81		22.38	23.00	5.98/6.6
122	Peroxiredoxin-2	PRDX2_BOVIN/22095988	18%	100%	120	21.93	21.93	23.70	5.37/5.25
	Cell structure								
13	Actin, aortic smooth muscle	ACTA_BOVIN/124007203	11%	100%	66	42.03	41.99	40.00	5.24/5.58
15	Actin, aortic smooth muscle	ACTA_BOVIN/124007203	8%	100%		41.99		49.00	5.24/6.43
17	Desmin	DESM_BOVIN/2959452	27%	100%	210	53.30	52.53	59.00	5.21/5.29
18	Desmin	DESM_BOVIN/2959452			224		52.53	58.50	5.21/5.32
22	Actin, aortic smooth muscle	ACTA_BOVIN/124007203	5%	99%		42.03		48.00	5.24/5.71
25	Actin, alpha cardiac muscle 1	ACTC_BOVIN/124007203			84		41.99	46.00	5.23/5.25
51	F-actin-capping protein subunit beta	CAPZB_BOVIN/11131728	8%	100%	83	31.30	33.72	33.50	6.01/5.25
60	Actin, alpha skeletal muscle	ACTS_BOVIN/62287933			54		42.02	27.50	5.23/5.57
66	Actin, aortic smooth muscle	ACTA_BOVIN/124007203	8%	100%	51.00	41.99	42.02	25.50	5.24/5.53

Table 11. Protein identification and function from *Longissimus dorsi* biopsies of beef cows (continued)

Gel No	Name	SWISS-PROT/NCBI accession number	Orbitrap S. cov.	Orbitrap Prob.	MALDITOF M. score	Theort. MW (KDa) Orb.	Theort. MW (KDa) MALD.	Experimental MW (KDa)	Theoretical /Exp. pI
	Contractile apparatus								
23	Tropomyosin beta chain	TPM2_BOVIN/75040654	31%	100%	231	32.82	32.82	39.00	4.66/4.65
24	Tropomyosin alpha-1 chain	TPM1_BOVIN/75052861	30%	100%	205	32.68	32.68	35.20	4.69/4.7
24	Tropomyosin beta chain	TPM2_BOVIN/75040654	11%	100%		32.82		35.20	4.66/4.73
27	MYH1 protein	Q05B72_BOVIN/115545466			64		34.02	39.00	5.23/6.08
33	Troponin T fast skeletal muscle	TNNT3_BOVIN/21039002			59		30.68	36.00	5.99/6.52
39	Troponin T fast skeletal muscle	TNNT3_BOVIN/21039002	10%	100%	60	32.11	30.68	35.60	5.99/6.56
42	Troponin T, slow skeletal muscle	TNNT1_BOVIN/66774017	10%	100%	34	31.27	31.27	34.30	5.71/5.76
43	Troponin T, slow skeletal muscle	TNNT1_BOVIN/66774017	14%	100%	53	31.27	31.27	34.30	5.71/5.86
44	Troponin T, slow skeletal muscle	TNNT1_BOVIN/66774017			50		31.27	35.10	5.71/6.02
47	Troponin T, slow skeletal muscle	TNNT1_BOVIN/66774017			50		31.27	32.50	5.71/6.39
62	Myosin light chain 1, skeletal muscle isoform	MLE1_BOVIN/115304798	56%	100%	45	20.91	19.49	23.00	4.96/5.02
82	Troponin T fast skeletal muscle	TNNT3_BOVIN	6%	100%		32.11		19.50	5.99/4.99
87	Cofilin-2	COF2_BOVIN/118572238	11%	100%		18.72		16.50	7.88/5.90
95	Myosin regulatory light chain 2	MLRV_BOVIN/122142995	30%	100%	103	18.96	18.97	18.00	4.86/4.68
96	Myosin regulatory light chain 2	MLRV_BOVIN/122142995	34%	100%		18.96		18.20	4.86/4.68
100	Myosin light chain 1, skeletal muscle isoform	MLE1_BOVIN/1181841	36%	100%	66.00	20.91	18.67	11.50	4.96/4.58
115	Myosin light chain 1, skeletal muscle isoform	MLE1_BOVIN/115304798	26%	100%		20.91		23.10	4.96/4.87
124	Myosin light chain 6B	MYL6B_HUMAN/109939949	6%	100%	125	22.75	23.39	21.50	5.56/5.71
	Other								
1	Serum albumin	ALBU_BOVIN/1351907			82		69.25	70	5.60/5.89
4	Fibrinogen alpha chain	FIBA_BOVIN/93141264	5%	100%		67.00		67.00	7.73/6.28
5	Serotransferrin	TRFE_BOVIN/2501351	4%	100%		77.74		69.00	6.50/6.5
7	Fibrinogen alpha chain	FIBA_BOVIN/93141264	14%	100%		67.00		64.00	7.73/6.75
15	Serum albumin	ALBU_BOVIN/1351907	8%	100%		69.28		50.00	5.60/6.43
19	Chain A, Crystal Structure Of Bovine Heart Mitochondrial	/114793901 BOVIN			65	49.18		53.80	5.46/5.77
20	Serum albumin	ALBU_BOVIN/1351907	4%	100%		69.28		50.00	5.60/5.82
22	Serum albumin	ALBU_BOVIN/1351907	6%	100%		69.28		49.00	5.60/5.61
22	T-complex protein 1 subunit theta	TCPQ_BOVIN/115305834			68		59.57	59.00	5.40/5.61
36	Fibrinogen gamma-B chain	FIBG_BOVIN/6980826	9%	100%	61	50.23	46.55	58.00	5.47/5.55
41	Four and a half LIM domains protein 3	FHL3_BOVIN/122140788	6%	100%		31.80		35.40	5.67/5.69
59	Prohibitin	PHB_BOVIN/88909243	8%	100%	49	29.80	29.79	29.40	5.57/5.57
75	Protein DJ-1	PARK7_BOVIN/75040204	19%	100%		20.02		21.50	6.84/6.35
80	Protein DJ-1	PARK7_BOVIN/75040204	18%	100%		20.02		21.50	6.84/5.99
92	Chain B, Crystal Structure Of H2o2 Treated Cu,Zn-Sod	/197724998 BOVIN			58		15.54	12.20	5.86/6.18
99	MYL1 protein	Q08E10_BOVIN/115304798			74		19.49	11.50	4.73/4.52
101	MYL1 protein	Q08E10_BOVIN/115304798			68		19.49	11.50	4.73/4.63
103	Myoglobin	MYG_BOVIN/127638	26%	100%	114	17.06	17.07	14.50	6.97/7.03
104	Chain S, The Crystal Structure Of Modified Bovine Fibrinogen	/6980826 BOVIN			117		46.55	59.00	5.49/5.40

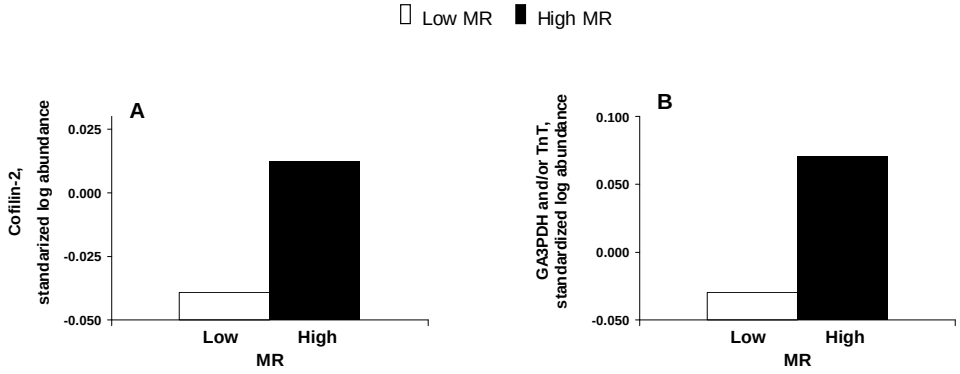

Figure 14. Protein abundance (standardized log abundance) of cofilin-2 (A, $P = 0.11$) and glyceraldehyde-3-phosphate dehydrogenase (GA3PDH) and/or troponin T fast skeletal muscle (TnT; B; $P = 0.16$), of beef cows with Low (n = 8) or High (n = 8) maintenance energy requirements (MR, NEm, Kcal·$BW^{-0.75}$·d^{-1}). Groups were defined as Low (> 0.5 SD less than mean) or High (> 0.5 SD more than mean). Standard errors averaged across treatments were 0.07 and 0.05 for cofilin-2 and glyceraldehyde-3-phosphate dehydrogenase and/or troponin T fast skeletal muscle, respectively.

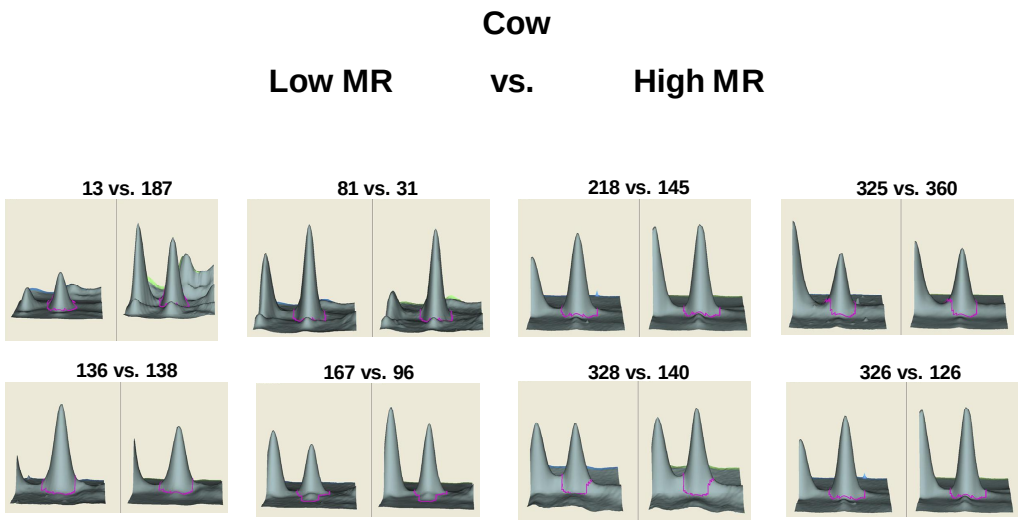

Figure 15. 3-D image of glyceraldehyde-3-phosphate dehydrogenase for beef cows with Low or High maintenance energy requirements (MR). Cow ID number on top of the image. Groups were defined as Low (> 0.5 SD less than mean) or High (> 0.5 SD more than mean). Image from low MR (n = 8) on the left of each pair and image from high MR (n = 8) on the right of each pair.

CHAPTER V

RELATIONSHIP OF RUMINAL TEMPERATURE WITH PARTURITION AND ESTRUS OF BEEF COWS

ABSTRACT: Angus x Hereford spring-calving cows (n = 30) were used to evaluate changes in ruminal temperature (RuT) related to parturition and estrus. Cows were synchronized and AI to a single sire. Temperature boluses (SmartStock®, LLC) were placed in the rumen at 7.0 ± 0.2 mo of gestation. Boluses were programmed to transmit RuT every 15 min. Cows (BW = 623 ± 44 kg, BCS = 4.9 ± 0.4) calved during 3 wk, and estrus was synchronized at 77 ± 7 d after calving with $PGF_{2\alpha}$. Cows were observed every 12 h to detect estrus. Daily average ambient temperatures ranged from 2 to 22 °C during parturition (February - March) and 17 to 25 °C during estrus (May - Jun). Ruminal temperature from 7 d before to 3 d after parturition and from 48 h before to 48 h after visual detection of estrus was analyzed using the MIXED procedure (SAS Inst., Inc., Cary, NC). Ruminal temperatures < 37.72 °C were attributed to water consumption and excluded from analyses. Day did not influence (P = 0.36) RuT from d 2 to 7 before parturition (38.94 ± 0.05 °C). Ruminal temperature decreased (P < 0.001) from d -2 to d -1 before parturition (38.88 ± 0.05 to 38.55 ± 0.05 °C, respectively). Ruminal temperature was not influenced (P = 0.23) by day from the day before parturition to 3 d after parturition (38.49 ± 0.05 °C). Ruminal temperature at 0 to 8 h after estrus was first

observed (38.98 ± 0.09 °C) was greater ($P < 0.001$) compared with RuT at the same daily h the day before (38.37 ± 0.11 °C) or the day after estrus (38.30 ± 0.09 °C). Ambient temperature did not influence ($P > 0.3$) RuT at parturition or estrus. Ruminal temperature significantly decreased the day before parturition and increased at estrus in spring-calving beef cows and has potential use to predict parturition and estrus.

Key Words: beef cows, estrus, parturition, temperature.

INTRODUCTION

Body temperature is related to physiological functions such as parturition and estrus in mammals. Weber (1910) observed a decrease in rectal temperature 28 h before parturition; decreases in body temperature before parturition range from 0.4 to 1.0 ºC (Wrenn et al., 1958; Lammoglia et al., 1997; Aoki et al., 2005). The decrease in body temperature before parturition is positively correlated with serum progesterone and these changes have been proposed as indicators of parturition (Birgel et al., 1994). Dystocia is a problem in cattle (Bazer and First, 1983), and calf deaths could be partially prevented by obstetrical assistance to cows (Bellows et al., 1987). Prevention of dystocia requires frequent observations or the use of a system to predict parturition. Prediction of parturition will allow supervision of cows and newborn calves and decrease loses.

Accurate estrous detection is a challenge for efficient AI. Calving interval, milk production, and hence profitability, are effected by estrous detection of dairy cattle (Pecsok et al., 1994; Maatje et al., 1997). Rate of genetic improvement of beef herds can be increased with the use of AI. Inadequate estrous detection and intense labor required for successful detection are some of the reasons why AI is not a common practice in beef production (Kyle et al., 1998). Vaginal temperature increases 0.3 to 0.8 °C during estrus

in dairy (Bobowiec et al., 1990; Redden et al., 1993; Fisher et al., 2008) and beef cows (Kyle et al., 1998). Maximum vaginal temperature is correlated with the onset of standing estrus (Rajamahendran et al., 1989) and the LH surge in dairy cows (Mosher et al., 1990; Rajamahendran and Taylor, 1991; Fisher et al., 2008). Vaginal temperature at estrus is increased for 4 to 11 h in dairy (Mosher et al., 1990; Redden et al., 1993; Fisher et al., 2008) and beef cows (Kyle et al., 1998).

Systems which utilize body temperature changes to predict parturition or estrus are not commercially available because technology required to record temperature has not been adequately developed (Rorie et al., 2002). Attached vaginal probes (Aoki et al., 2005), implanted electronic temperature monitors (Lammoglia et al., 1997), and inserted vaginal devices (Redden et al., 1993; Kyle et al., 1998; Fisher et al., 2008), are invasive and require special attention to ensure proper function. An automated, frequent data collection system to determine temperature of cows must be practical, minimally invasive, and accurate to record body temperatures that can be associated with parturition and estrus.

Recent development of ruminal boluses allows frequent data collection in real time, with minimal impact on animal handling and behavior. SmartStock® ruminal boluses can be programmed to record temperature at various time intervals (SmartStock®, personal communication). Frequent recording of ruminal temperature may be required to detect the increase in body temperature during estrus. The objective of this study was to evaluate changes in ruminal temperature associated with parturition and estrus in spring-calving beef cows.

MATERIALS AND METHODS

Animals and management

The Oklahoma State University Animal Care and Use Committee approved all the experimental procedures used in this study. Estrous cycles of Angus x Hereford multiparous spring-calving beef cows (n = 30; 5 to 8 yr of age) were synchronized and cows were AI with semen from a single Angus sire. During the last trimester of gestation, cows grazed native prairie pasture (*Andropogon scoparius, Andropogon gerardii*) and received 1.4 kg of a 38% CP supplement. Ruminal boluses (SmartStock®, LLC, Pawnee, OK), were orally inserted into the rumen of each cow, using a custom balling gun at 7.0 ± 0.2 month of gestation. The ruminal bolus data collection system (SmartStock®, LLC) consisted of four components: (a) radio frequency ruminal temperature sensor bolus (8.25 cm x 3.17 cm; 114 g), (b) an antenna in the cow pen for data collection from boluses, (c) a receiver antenna for transmitted data, and (d) a personal computer with software for data storage. The data collection and the receiver antennas were within 100 m. Date, time, cow identification and ruminal temperature (every 15 min) were transmitted by radiotelemetry and stored in the computer for analyses. Cows weighed 623 ± 44 kg, and had a BCS of 4.9 ± 0.4 prior to parturition. From 10 d before expected parturition to 7 d after parturition (February – March), cows and calves were maintained in a pen (60 x 80 m). After parturition cows were fed 1.8 kg of a 38% CP supplement and had water and prairie hay ad libitum. Time of parturition was recorded within 6 h. Ruminal temperatures were recorded from 7 d before to 3 d after parturition.

Estrous cycles of cows (n = 21, BW = 545 ± 35 kg, BCS of 4.8 ± 0.4) were synchronized at 77 ± 7 d after calving (May to June) with $PGF_{2\alpha}$ (25 mg of Lutalyse i.m.; Pfizer Inc., NY). Cows that did not exhibit estrus after treatment were retreated with $PGF_{2\alpha}$ at 14 d after the first treatment. Chalk (ALL-WEATHER® PAINTSTIK® LA-CO Industries Inc., IL) was applied to the tailhead of each cow at $PGF_{2\alpha}$ treatment. Cows were maintained in a pen (60 x 80 m) and were observed for 30 min at 0700 and 1900 h to detect estrus. Cows were considered in estrus if they stood to be mounted by another cow and the chalk present 12 h before was rubbed off the tailhead. Cows were AI 12 h after detection of estrus. Ruminal temperatures were collected from 48 h before to 48 h after a cow was first observed in estrus. Pregnancy was confirmed by ultrasonography 29 ± 1d after AI. Ambient temperature data were collected from the Mesonet (site Marena; www.mesonet.org).

Statistical analyses

Ruminal temperatures were analyzed using MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) to evaluate repeated measurements for a cow. Ruminal temperatures < 37.72 ºC (10 to 12% of data) were considered a consequence of water consumption and were excluded from final analyses and reported results. However, analyses conducted with all data or with the exclusion of temperatures < 37.72 ºC resulted in similar results (data not shown). Ruminal temperatures were normally distributed and excluding values < 37.72 ºC reduced skewness and variance. Average daily ambient temperature was included in all models as a covariable. Effects that were not significant ($P > 0.4$) were eliminated from the final model. Six covariance structures (variance component, compound symmetry, Huynh-Feldt, first-order autoregressive, Toeplitz and unstructured)

were examined to identify and use the best structure according to the goodness of fit statistic. Variance components for all analyses were estimated using the restricted maximum-likelihood method. The covariance structure with the best goodness of fit statistics was the first-order autoregressive for all analyses. The Kenward-Roger procedure was used to determine the denominator degrees of freedom.

The statistical model for diurnal variation in RuT included hour with average daily ambient temperature as a covariable. Average daily ambient temperature effect on diurnal variation of ruminal temperature was retained in the final model for parturition ($P = 0.39$) and deleted ($P = 0.82$) from the final model for estrus.

Mean daily ruminal temperatures were calculated at parturition for each cow. The statistical model included day relative to parturition with average ambient temperature as a covariable. Daily ambient temperature ($P = 0.32$) was retained in the model.

Evaluation of ruminal temperature at estrus included several comparisons. Initially, the model included hour relative to first observed estrus with average daily ambient temperature as a covariable; average daily ambient temperature effect was not significant ($P = 0.83$), and was deleted from the model. Evaluations of ruminal temperature at different periods were made. Mean ruminal temperature for selected periods per cow was calculated for comparisons. Periods were: from 8 h before to 8 h after estrus was first observed, from 4 h before to 4 h after estrus was first observed, and from the time estrus was first observed (h 0) to 8 h after (Figure 16). Periods to compare RuT at estrus were selected considering characteristics of estrus, methods of detection and diurnal RuT. Mean duration of estrus is about 6 h in suckled beef cows (Ciccioli et al., 2003), and 16 h in nonlactating beef cows (White et al., 2002). Vaginal temperature

decreases during proestrus, increases during estrus, and decreases again after estrus in dairy and beef cows (Bobowiec et al., 1990; Kyle et al., 1998). With visual detection of estrus, cows may be first observed in estrus at 1 min or 11 h and 59 min after the onset of estrus. Ruminal temperature had diurnal variation, so times (periods ≤ 16 h) during which RuT were compared before, during and after estrus included the same daily hours commencing 24 h before and after selected period around estrus. Mean ruminal temperature for a period was compared with mean ruminal temperature for the same daily hours the day before or the day after estrus. The statistical model for ruminal temperature at estrus included period. The period with the maximum increase in ruminal temperature at estrus was then compared with the average ruminal temperature on the same daily hours of the previous 2 d.

Ruminal temperature at estrus was further analyzed by adjusting ruminal temperature during visual detection of estrus to the hour of maximum ruminal temperature (8 to 19 observations per h). The statistical model included hour of maximum ruminal temperature, with two covariables: daily hour ($P = 0.15$), and average daily ambient temperature ($P = 0.09$).

RESULTS

One cow had ruminal temperatures greater than 39.5 ºC for 6 d during the trial, was diagnosed as sick, and was excluded from analyses. All cows had normal parturitions and healthy single calves. Technical difficulties recording ruminal temperatures resulted in approximately 12 h of missing data per cow during the 7 d before to 3 d after parturition and two d before and after estrus. As a consequence, only cows with recorded ruminal temperatures on 4 to 23 different daily hours (6 to 85

observations) were included in the analyses for parturition resulting in data from 20 to 29 cows per day. For RuT at estrus analyses, only cows with records from 4 to 16 different daily hours (4 to 64 observations) were included in the analyses resulting in 12 to 21 cows per period.

There was diurnal variation in RuT during parturition and estrus. Minimal and maximal daily RuT from 7 d before to 3 d after parturition occurred at 1130 h (38.5 ± 0.06 ºC) and at 2045 h (39.01 ± 0.07 ºC), respectively, when individual RuT less than 37.72 ºC were deleted (Figure 17). Minimal and maximal temperatures 48 h before and after estrus occurred at 1115 h (38.22 ± 0.06 ºC) and at 2115 h (38.78 ± 0.07 ºC), respectively, when individual RuT less than 37.72 ºC were deleted (Figure 18). In both cases, RuT decreased after 0700 h to the minimal at mid-day, then temperatures increased from 1230 h to maximums around 2100 h.

Figure 19 depicts RuT for an individual cow at 3 d after parturition. At 0915 h, RuT decreased 5 ºC, indicative of a water consumption event. Ruminal temperature was < 37.5 ºC within 30 min and > 38.00 ºC by 3 h after water consumption. A second water consumption event may have occurred at 1215 h when RuT decreased to 35.0 ºC. A third water consumption event may have occurred at 1700 h, when RuT decreased to 37.72 ºC. The decrease in RuT at 1900 h was for 15 min and may or may not have been associated with water consumption.

Parturition

Mean RuT, from 7 d before to 3 d after parturition, was 38.75 ± 0.01 ºC. Ruminal temperature was effected by day relative to parturition ($P < 0.001$; Figure 20). Daily RuT did not differ ($P = 0.36$) from -7 to -2 d (38.94 ± 0.05 °C) before parturition (d 0). Daily

RuT decreased ($P < 0.001$) from -2 to -1 d before parturition (38.88 to 38.55 ± 0.05 °C, respectively). Daily RuT did not differ ($P = 0.23$) from the day before parturition to 3 d after parturition (38.49 ± 0.05 °C).

During parturition, daily average ambient temperatures ranged from 2 to 22 ºC, and daily minimum and maximum ambient temperatures ranged from -8 to 18 and 6 to 27 ºC, respectively. Daily average ambient temperature as a covariable did not influence RuT during parturition ($P = 0.32$).

Estrus

Cows exhibited estrus within 5 d after $PGF_{2\alpha}$ treatment. Eighteen cows were in estrus after the first treatment, and 3 cows were treated twice with $PGF_{2\alpha}$ to induce estrus. Percentages of cows observed in estrus on d 2, 3, 4, and 5 after the first $PGF_{2\alpha}$ treatment were: 29, 29, 14, and 14%, respectively. Nine of the cows detected were first observed in estrus in the morning, and 12 were first observed in the afternoon.

Mean daily RuT (n = 21) during 48 h before and after estrus was first observed was 38.54 ± 0.01 ºC. Ruminal temperature was greater ($P < 0.001$) from 8 h before to 8 h after estrus was first observed compared with RuT the same daily hours the day before or day after estrus (Table 12). Similarly, RuT was greater ($P < 0.001$) from 4 h before to 4 h after estrus was first observed compared with RuT the same daily hours the day before or the day after estrus, and RuT was greater from 0 h to 8 h after estrus was first observed compared with RuT the same daily hours the day before or day after estrus. Mean ruminal temperature was 0.44 ºC greater ($P < 0.001$) during 8 h before to 8 h after estrus was first observed compared with the same hours the previous day; RuT was 0.52 ºC greater ($P < 0.001$) during 4 h before to 4 h after estrus was first observed, compared with

the same hours the previous day. Ruminal temperature was 0.61 ºC greater ($P < 0.001$) during 0 to 8 h after estrus was first observed compared with the same hours the previous day (Figure 21). Ruminal temperature was greater ($P < 0.001$) during the first 8 h after cows were first observed in estrus (38.98 ± 0.10) compared with RuT on the same daily hours the two previous days (38.45 ± 0.10).

Ruminal temperature was greater ($P < 0.05$) from 2 h before to 2 h after maximum RuT compared with -3 to -10 h and 6 to 10 h relative to maximum RuT associated with estrus (Figure 22). The maximum RuT associated with estrus (h 0) was 39.88 ± 0.11 ºC, and occurred 1.8 ± 6.3 h before estrus was first observed (data not shown).

During collection of estrous data, daily average ambient temperatures ranged from 17 to 25 ºC, and daily minimum and maximum ambient temperature ranged from 11 to 21 and 22 to 31 ºC, respectively. Daily average ambient temperature as a covariable did not influence RuT during estrus ($P = 0.83$). Eightysix percent of the cows inseminated at the synchronized estrus were confirmed pregnant by ultrasound 30 d after AI.

DISCUSSION

Diurnal variation in RuT observed in beef cows in late gestation and at estrus in this study was also observed in steers (Dye, 2005). Similar to the current study, minimal RuT (38.90 ºC) occurred from 0900 to 1100 h and maximum temperatures (39.3 ºC) were at 2100 to 0200 h in steers, and RuT were positively associated with rectal temperature ($R^2 = 0.80$; Dye, 2005). Diurnal variation in RuT may be associated with water consumption. The decrease in RuT at mid-day was greater if all data were included. In

beef steers (Dye, 2005) and sheep (Brod et al., 1982), decreases in RuT were observed after water consumption and the magnitude of the decrease was dependent on the volume and temperature of the consumed water. Ruminal temperatures in dairy cows decreased as much as 8.5 ºC after intake of cold water (Bewly et al., 2008). Volume and temperature of the consumed water effected the magnitude of the decrease and the duration of time for the RuT to return to the pre-drinking temperature (Bewely et al., 2008). When cows consumed water that was similar in temperature to body temperature, a decrease in 0.4 ºC and a rapid (15 min) return to pre-drinking RuT occurred (Bewely et al., 2008). In the current experiment, water temperatures were always less than body temperatures (15 – 20 ºC) and decreases in RuT were very dramatic especially at mid-day. Time of water consumption was not determined in this experiment so the effect of water consumption on RuT cannot be determined.

The decrease in RuT at parturition and the increase in RuT at estrus strongly support the concept of body temperature changes associated with parturition (Lamoglia et al., 1997; Aoki et al., 2005) and estrus (Redden et al., 1993; Kyle et al., 1998; Fisher et al., 2008). Similar to the decrease in RuT the day before parturition, a decrease in body temperature (as much as 0.66 ºC) occurred about 2 d before parturition in dairy (Wrenn et al., 1958; Ewbank, 1963; Aoki et al., 2005) and beef cows (Lammoglia et al., 1997). Vaginal temperature decreased 0.5 ºC 1 or 2 d before parturition in dairy cows (Wrenn et al., 1958). In other experiments with cows, rectal temperature decreased 0.61 ºC at 54 h before parturition (Ewbank, 1963), and body temperature decreased 0.66 ºC at 16 h before parturition (Lammoglia et al., 1997).

Ambient temperature (16.5 to 26 ºC) influenced body temperature from 56 to 144 h but not 8 to 48 h before parturition in beef cows (Lammoglia et al., 1997). However, cooler ambient temperatures (5.5 to 22.9 ºC) did not influence vaginal temperatures of cows during 6 d before parturition (Aoki et al., 2005). In the present study, average ambient temperatures at parturition ranged from 2 to 20 ºC, and did not influence RuT. Ambient temperature could have a greater impact on temperature of the *Obliquus internus abdominis* muscle of the flank of the cows (Lammoglia et al., 1997) compared with temperature in the vagina or the rumen which are deeper in the body.

Metabolic adaptation and endocrine and behavioral changes during the peripartum period may cause the decrease in RuT prior to parturition. Greater body temperatures during the last week of pregnancy, a decrease in temperature one to 2 d prior to parturition (Wrenn et al., 1958; Lammoglia et al., 1997; Aoki et al., 2005), and the correlation among progesterone in plasma and body temperature (Birgel et al., 1994), indicates a thermogenic effect of progesterone (Wrenn et al., 1958). Vaginal temperature increased in ovariectomized cows treated with progestagens compared with untreated cows (Wrenn et al., 1958). Greater vaginal temperatures during the luteal phase of the estrous cycle, and reduced temperatures before and after estrus in cows (Bowiec et al., 1990; Kyle et al., 1998) support the hypothesis of the thermogenic effect of progesterone.

Changes in concentrations of progesterone, estradiol-17β, thyroxine (T_4), triiodothyronine (T_3), and $PGF_{2\alpha}$ in plasma, were associated with the decrease in body temperature prior to parturition in cows (Lammoglia et al., 1997), and 30% of the variation in the decrease in body temperature was accounted for changes in concentrations of these hormones at parturition (Lammoglia et al., 1997). The hormones

with greater impacts on body temperature at parturition were T_3 and $PGF_{2\alpha}$, (Lammoglia et al., 1997). Concentrations of T_4 in serum of Holstein heifers were constant from 90 to 260 d of gestation (Hernandez et al., 1972). Reduced feed and water intake 3 wk before parturition (Grummer et al., 2004; Lukas et al., 2008), may have an impact on the thyroid, and on heat generated in the rumen. Plasma concentrations of T_4 decreased during feed restriction and increased with realimentation of nonlactating beef cows (Richards et al., 1995). Feed intake (FI) is associated with greater concentrations of T_4 in plasma of suckled postpartum beef cows (Ciccioli et al., 2003) and steers (Hersom et al., 2004). Heat production decreased at 7 d after feed restriction in nonpregnant nonlactating cows, adaptation to energy availability may occur by decreasing heat production during restriction (Freetly et al., 2006). Decreased FI prior to parturition may reduce concentrations of thyroid hormones in plasma and hence reduce heat production. In addition, a decrease in FI may decrease the heat production associated with ruminal fermentation. Ruminal temperature of gestating beef cows increased 0.9 °C at 8 h after consumption of a complete maintenance diet (NRC, 1996; unpublished, 2008).

This is the first report of an increase in RuT associated with visually detected estrus in beef cows. Ruminal temperature was greater during 8 or 16 h periods at estrus compared with RuT the same daily hours the day before or the average for the previous 2 day. An increase of 0.61 ºC was observed during 8 h after estrus was first detected with twice daily observations compared with the same daily hours on the previous day, and RuT was decreased during the same daily hours the day after estrus. Vaginal temperature increased 0.6 ºC at estrus in lactating dairy cows (Redden et al., 1993) and 0.90 ºC in lactating beef cows (Kyle et al., 1998). Vaginal temperature increased at least 0.3 ºC

during estrus compared with the average of the previous 3 d in dairy and beef cows (Clapper et al., 1990; Mosher et al., 1990; Kyle et al., 1998).

Ruminal temperature, adjusted to the maximum at estrus (h 0), increased for 4 h at estrus in this study. Vaginal temperature increased during estrus for 11 h in dairy heifers (Mosher et al., 1990), and the duration of the increase in vaginal temperature at estrus was 4 to 8 h in dairy (Clapper et al., 1990; Redden et al., 1993; Fisher et al., 2008) and beef cows (Kyle et al., 1998). Duration of the increase in body temperature during estrus may vary dependent on equipment used, frequency of determination, environmental conditions, and physiological state of females. If body temperature is only recorded once a day it may not be possible to identify changes associated with estrus.

Average ambient temperatures during our study were similar to temperatures within the thermoneutral zone for beef cows (Hahn, 1999). Similar to other studies (Kyle et al., 1998; Piccione et al., 2003), RuT at estrus was not influenced by ambient temperature. Rectal temperature increased during estrus when average ambient temperatures ranged from 9 to 15 ºC in dairy cows (Piccione et al., 2003) and from -5.2 to 6.7 ºC in beef cows (Kyle et al., 1998). Vaginal temperatures when recorded only once a day were influenced more by ambient temperature than by day of the estrous cycle and are not useful to predict estrus (Lewis and Newman, 1984).

An increase in RuT at estrus may be associated with behavioral and endocrine changes. Physical activity of dairy cows increase at estrus (Kiddy, 1977; Pennington et al., 1986), and is correlated with rectal temperature in dairy cows (Walton and King, 1986). An increase in physical activity of steers is associated with an increase in tympanic temperature (Mader et al., 2005). Decreased water intake during estrus (Meyer

et al., 2004; Lukas et al., 2008) may influence body temperature. Temperature recorded in the ear was decreased after cold water was consumed of dairy cows (Stermer et al., 1986). Water consumption decreases RuT in sheep (Brod et al., 1982), steers (Dye, 2005) and dairy cows (Bewley et al., 2008; Ipema et al., 2008). Voluntary FI changes during estrus are not established; FI may decrease (Diskin and Sreenan, 2000), remain constant (De Silva et al., 1981) or increase (Lukas et al., 2008) during estrus. The impact of feed and water intake on RuT are not well defined.

Endocrine changes before, during, and after estrus may impact body temperature of cows and concentrations of progesterone in plasma during the estrous cycle have been associated with vaginal temperature (Wrenn et al., 1958). Vaginal temperature is greater during the luteal phase compared with the follicular phase of the estrous cycle, except for the increase in vaginal temperature at estrus (Bobowiec et al., 1990; Kyle et al., 1998). Increased estradiol during estrus may have an impact on body temperature. Treatment of ovariectomized dairy cows with estradiol-17β increased uterine blood flow (Roman-Ponce et al., 1978). Uterine blood flow increased during estrus in the sheep (Roman-Ponce et al., 1983), cows (Bollwein et al., 2000) and mares (Bollwein et al., 2002). The increase in UBF during 48 h before to 24 h after estrus was first observed (visual detection every 12 h) was negatively associated with concentrations of progesterone in plasma and positively associated with the rates of estradiol and estrone to progesterone in sheep (Roman-Ponce et al., 1983). Treatment of ovariectomized dairy cows with estradiol-17β increased UBF (Roman-Ponce et al., 1978). Altered blood flow at estrus may be related to increased RuT at estrus.

IMPLICATIONS

Ruminal temperature of beef cows change before parturition and during estrus. Ruminal temperature is significantly decreased the day before parturition and is increased at estrus, and ruminal temperature may have potential to predict parturition and estrus. Methodology to measure ruminal temperature is minimally invasive, frequent records of real time data can be obtained, minimal labor is required, and cows can be in their natural environment. Additional studies to evaluate ruminal temperature associated with the estrous cycle and the use of ruminal boluses to predict parturition and estrus will result in technology to increase reproductive performance of beef cows.

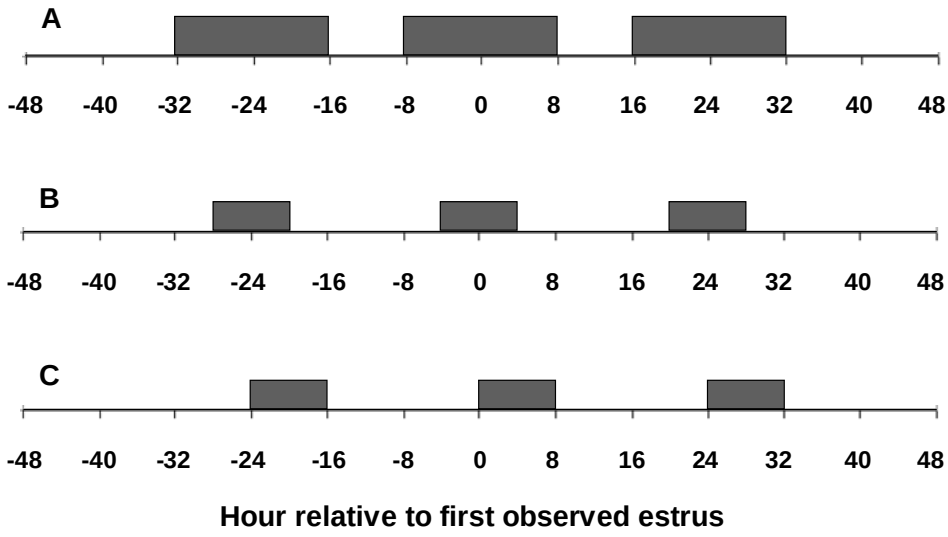

Figure 16. Time periods during which ruminal temperatures were compared relative to first observed estrus (h 0). **A**: Period from 8 h before to 8 h after estrus was first observed was compared with the same daily hours the day before (-32 to -16 h) and the day after (16 to 32 h). **B**: Period from 4 h before to 4 h after estrus was first observed was compared with the same daily hours the day before (-28 to -20 h) and the day after (20 to 28 h). **C**: Period from the time estrus was first observed (h 0) to 8 h after estrus was first observed was compared with the same daily hours the day before (-24 to -16 h) and the day after (24 to 32 h). Mean ruminal temperature per cow for selected periods were calculated for comparisons.

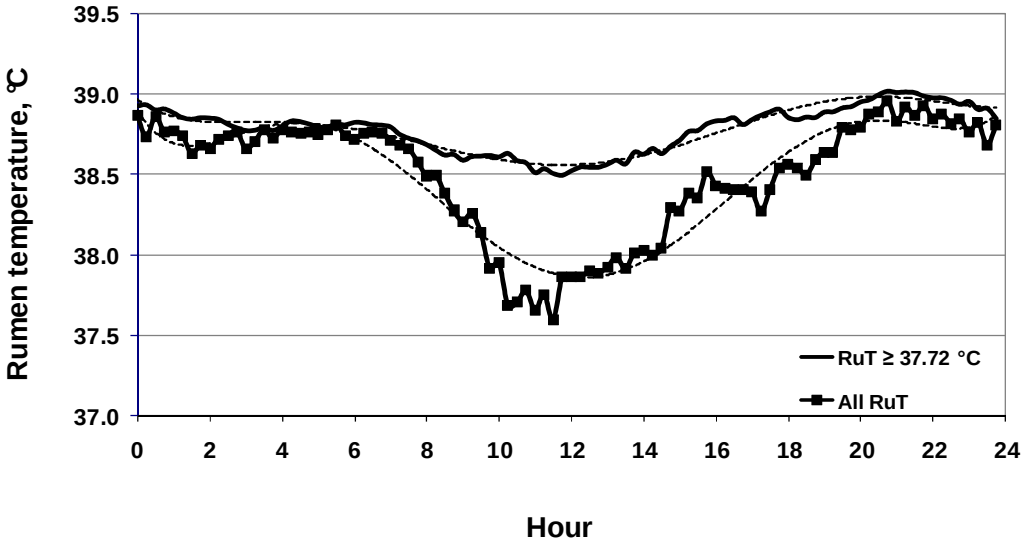

Figure 17. Diurnal variation in mean ruminal temperature (RuT) from 7 d before to 3 d after parturition of spring calving beef cows (n = 29) including all RuT or RuT ≥ 37.72 °C. Time trends are depicted by doted lines. Hour 12 equals 1200 h.

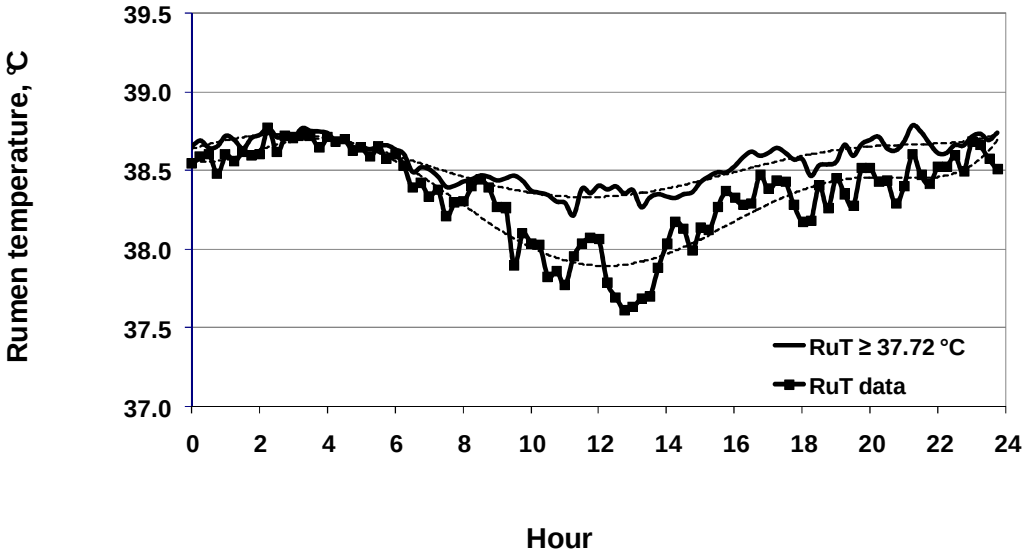

Figure 18. Diurnal variation in mean ruminal temperature (RuT) from 48 h before to 48 h after estrus of spring calving beef cows (n = 21) including all RuT or RuT ≥ 37.72 °C. Time trends are depicted by doted lines. Hour 12 equals 1200 h.

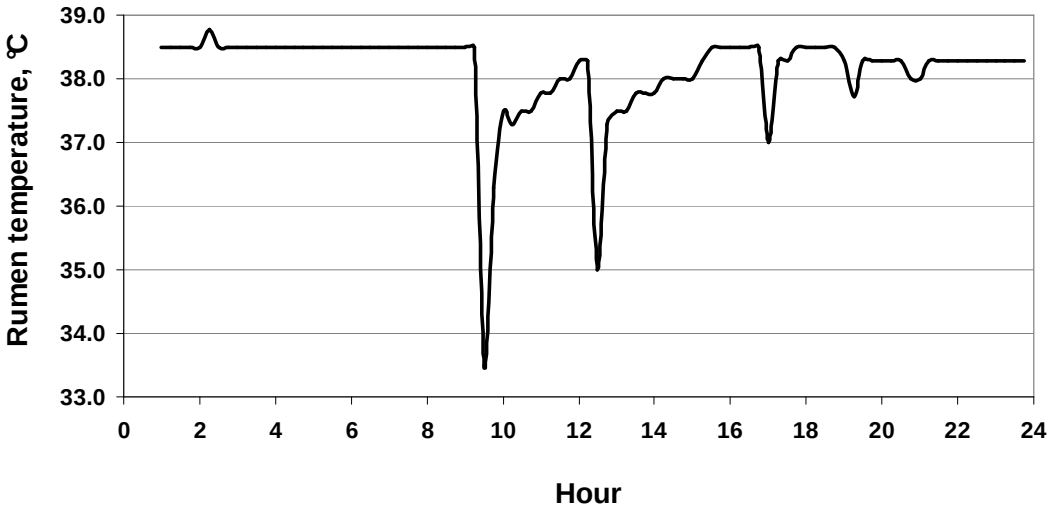

Figure 19. Ruminal temperature (RuT) of a single cow on d 3 after parturition including all RuT. Hour 12 equals 1200 h.

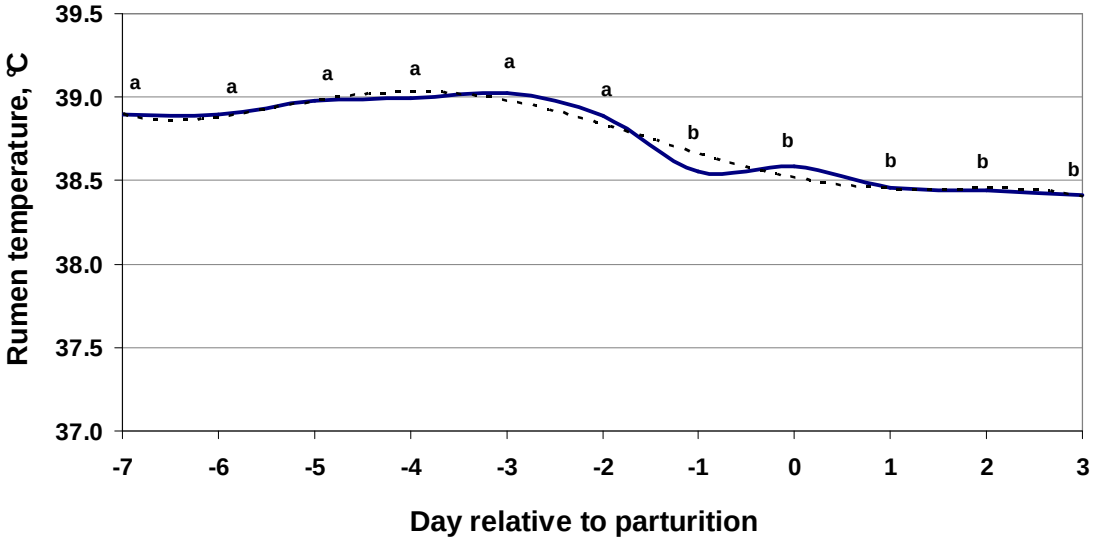

Figure 20. Mean ruminal temperature (RuT) by day relative to parturition (d 0) and trend line (doted line) of spring-calving beef cows (n = 29). Ruminal temperatures < 37.72 °C are excluded. [a,b] Means differ (P < 0.001). Standard errors averaged over days was 0.08.

Table 12. Mean ruminal temperature (RuT) of beef cows at different periods relative to first observed in estrus compared with the same daily hours the day before or the day after estrus

	Period[1]				
Item	d before estrus	around estrus	d after estrus	SEM	*P* value
Hours in the period	-32 to -16	-8 to 8	16 to 32		
Cows, No.	15	21	20		
RuT, ℃	38.23 [a]	38.67 [b]	38.04 [a]	0.1	< 0.001
Hours in the period	-28 to -20	-4 to 4	20 to 28		
Cows, No.	12	20	19		
RuT, ℃	38.5 [a]	39.02 [b]	38.23 [a]	0.09	< 0.001
Hours in the period	-24 to -16	0 to 8	24 to 32		
Cows, No.	12	17	16		
RuT, ℃	38.37 [a]	38.98 [b]	38.3 [a]	0.1	< 0.001

[1] Periods are hours relative to first observed estrus (h 0) including the same daily hours in three different days.

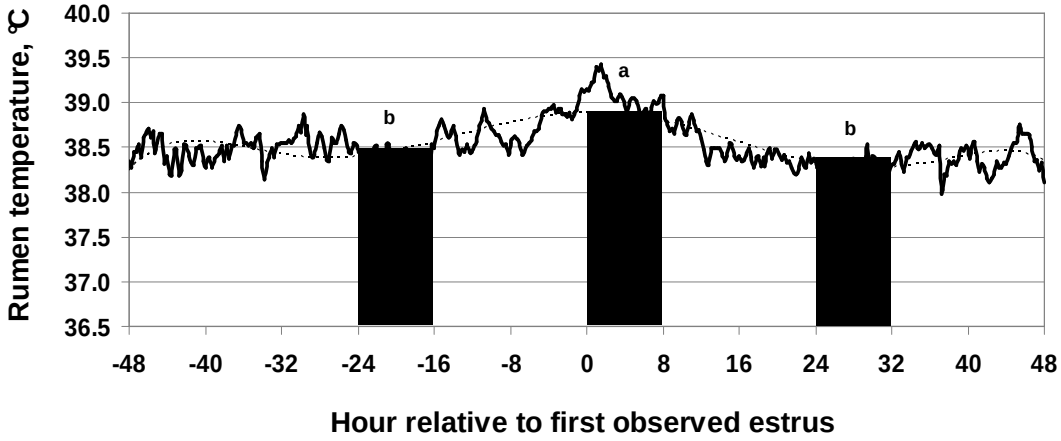

Figure 21. Mean ruminal temperature (RuT) relative to visual detection of estrus (h 0) and trend line (doted lines) of spring-calving beef cows. Ruminal temperatures < 37.72 °C are excluded. Bars represent mean RuT for a period of 8 h after visual detection of estrus (38.98 ± 0.09 °C, n = 17) and for the same daily hours the day before (38.37 ± 0.11 °C, n = 12) and day after (38.30 ± 0.10 °C, n = 16). [a,b] Means differ ($P < 0.001$) for the periods. Standard errors averaged over periods averaged 0.1.

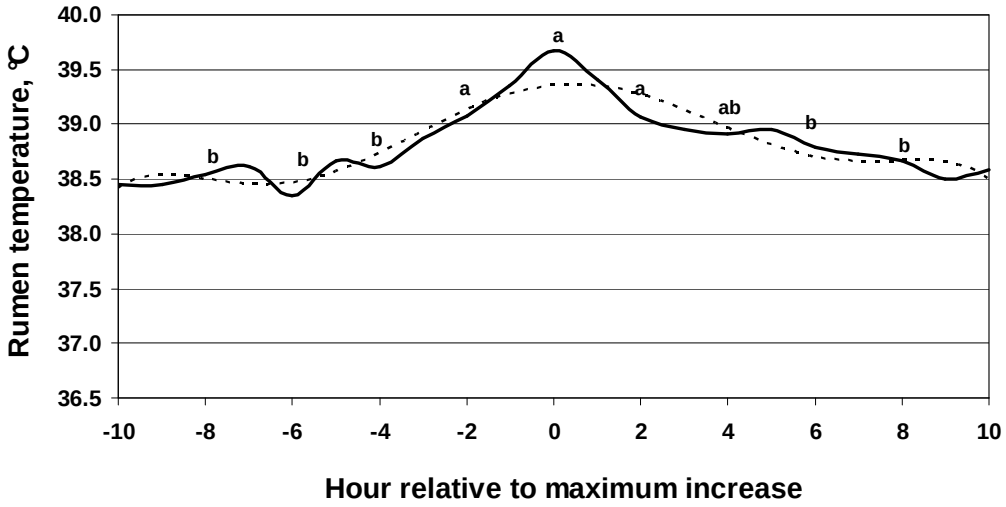

Figure 22. Ruminal temperature adjusted to the maximum at time 0 and trend line (doted lines) of spring-calving beef cows (n = 8 to 19 per h). Ruminal temperatures < 37.72 °C were excluded.[a,b] Mean with a different superscript differ ($P < 0.05$) . Standard errors averaged over hours was 0.13.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Effects of postpartum weight gain and treatment with bovine somatotropin (bST) on calf growth and concentrations of hormones in plasma of beef cows were evaluated. Angus x Hereford cows were stratified based on calving date and BCS at calving, and randomly assigned to a 2 x 2 factorial: weight gain (WG) $\leq$ 0.4 kg/d (M) or > 0.40 kg/d (H), and cows were injected with bST (250 mg) or saline (C) on d 31 and 45 post partum. Before treatment with bST, H had greater concentrations of IGF-I and glucose in plasma compared with M cows. Concentrations of IGF-I in plasma were greater in HbST compared with MbST, MC or HC on 3, 7 and 10 d, after the bST treatments. After the first bST treatment, concentrations of glucose in plasma were greater in HbST compared with HC, MC, and MbST. Concentrations of insulin in plasma were greater in H compared with M cows before bST treatment and during d 45 to 59 post partum. Weight gain and treatment with bST did not influence the percentage of cows with luteal activity by 59 d post partum or the subsequent calving interval. Average daily gain of calves to 140 d of age was greater for bST and H treated cows compared with C and M cows, respectively, and ADG to 220 d of age was greater for H compared with M cows. Weight gain of young lactating beef cows influenced plasma concentrations of IGF-I and

glucose after treatment with bST, and WG and bST treatment of cows influenced calf growth.

Angus x Hereford cows were used to evaluate variation in maintenance energy requirements (MR) and the influence of MR on cow and calf performance, plasma concentrations of IGF-I, T_4, glucose, and insulin, ruminal temperature, and abundance of different proteins in muscle. The proteome of the *Longissimus dorsi* muscle was also described. Maintenance energy requirements of Angus x Hereford cows were estimated in feeding trials on 3 consecutive years. Cows were classified based on MR as low (> 0.5 SD less than mean, LMR), moderate (± 0.5 SD of mean, MMR) or high (> 0.5 SD more than mean, HMR) MR within each year. Muscle biopsies were taken from LMR and HMR at maintenance in yr 2 and yr 3. Proteins from muscle were separated by two-dimensional, difference gel electrophoresis and abundance was quantified and compared. The greatest differences in MR for all cows were 29, 24, and 25% in yr 1, 2 and 3, respectively. Daily MR (NE_m, $Kcal \cdot BW^{-0.75} \cdot d^{-1}$) averaged 89.2 ± 6.3, 93.0 ± 4.9, and 90.4 ± 4.6, in yr 1, 2 and 3, respectively. Birth and weaning weights of calves, BW and BCS of cows, resumption of luteal activity, plasma concentrations of hormones, and ruminal temperature of the cows, were not influenced by MR. However, concentrations of IGF-I in plasma were negatively correlated with MR at 2 mo post partum. From a total of 103 isolated proteins, 52 gene products were identified. Protein abundance tended to be greater in HMR compared with LMR cows for cofilin-2. Greater abundance of cofilin-2 in HMR cows may have application as a biomarker for MR.

Changes in ruminal temperature (RuT) related to parturition and estrus were evaluated in Angus x Hereford spring-calving cows. Cows were synchronized and AI to

a single sire. Temperature boluses were placed in the rumen and programmed to transmit RuT every 15 min. Cows calved during 3 wk, and estrus was synchronized at 77 ± 7 d after calving with $PGF_{2\alpha}$. Cows were observed every 12 h to detect estrus. Day did not influence RuT from d 2 to 7 before parturition. Ruminal temperature decreased ($P < 0.0001$) from d -2 to d -1 before parturition and remained constant until d 3 after parturition. Ruminal temperature at 0 to 8 h after estrus was first observed was greater ($P < 0.001$) compared with RuT at the same daily h the day before or the day after estrus. Ruminal temperature significantly decreased the day before parturition and increased at estrus in spring-calving beef cows. These results indicate the rumen boluses have potential application to predict parturition and estrus.

In conclusion, different strategies to optimize reproductive performance and to efficiently utilize feed energy in the cow-calf segment of beef production have been evaluated. Increased nutrient intake and treatment of postpartum beef cows with bST increased plasma concentrations of IGF-I and glucose. However, such an early treatment did not influence the postpartum anestrous interval or the subsequent calving interval. Greater weight gain and treatment of cows with bST increased ADG of calves. The variation in maintenance energy requirements (MR) within a herd was confirmed, and was not associated with calf growth, and plasma concentrations T_4, glucose and insulin, or ruminal temperature in the cows. However MR were negatively correlated with concentrations of IGF-I in plasma of the cows under grazing conditions. In addition, Cofilin-2 abundance tended to be greater in cows with a greater MR and may have application as a biomarker for MR. The description of the proteome of *Longissimus dorsi* in beef cows for the first time, and the protocol developed to separate the proteins

provide important information for future research. Detection of biomarkers for MR will allow identification and selection of more efficient cows and therefore improve efficiency of production. We were able to determine that ruminal temperature decreases before parturition and increases at estrus. The methodology to measure ruminal temperature is minimally invasive, frequent records of real time data can be obtained, minimal labor is required, and cows can be in their natural environment. This study provides evidence that the use of rumen boluses to measure ruminal temperature has potential application in the beef cattle industry to predict parturition and estrus and to monitor health.

Opportunities exist to increase efficiency in the cow-calf segment of the beef industry. Weaning heavier calves using similar resources, or weaning of similar weight calves using less energy, will improve efficiency of beef cattle production and enhance sustainability of the environment.

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VITA

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Doctor of Philosophy

Dissertation: MAINTENANCE ENERGY REQUIREMENTS, POSTPARTUM REPRODUCTION, AND RUMINAL TEMPERATURE AT PARTURITION AND ESTRUS OF BEEF COWS

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Title of Study: MAINTENANCE ENERGY REQUIREMENTS, POSTPARTUM REPRODUCTION, AND RUMINAL TEMPERATURE AT PARTURITION AND ESTRUS OF BEEF COWS

Pages in Study: 174 Candidate for the Degree of Doctor of Philosophy

Major Field: Animal Breeding and Reproduction

Scope and Method of Study: Angus x Hereford cows were used to determine the effects of postpartum weight gain (WG) and treatment with bovine somatotropin (bST) on concentrations of hormones in plasma and calf growth. Cows were assigned to a 2 x 2 factorial: WG, to gain either ≤ 0.4 kg/d or > 0.40 kg/d until d 59 post partum. Cows were injected with bST (250 mg) or saline on d 31 and 45 after calving. Variation in maintenance energy requirement (MR) was determined in nonlactating, pregnant Angus x Hereford cows in feeding trials in each of 3 yr. Cows were classified based on MR as low (> 0.5 SD less than mean, L), moderate (± 0.5 SD of mean) or high (> 0.5 SD more than mean, H). Relationships among MR and calf performance, plasma concentrations of IGF-I, T_4, glucose, insulin and ruminal temperature were determined in yr 2; description of the proteome and evaluation of protein abundance in Longissimus dorsi (LM) of cows with different MR were evaluated in yr 2 and 3; and the relationship of ruminal temperature with parturition and estrus was evaluated. Proteins from LM were separated separate by 2D-DIGE and abundance was quantified and compared. Rumen boluses were placed in the rumen of the cows to measure ruminal temperature (every 15 min) around parturition and estrus.

Findings and Conclusions: Weight gain influenced plasma concentrations of IGF-I and glucose after treatment of cows with bST. However, treatment did not influence reproduction of the cows. Weight gain and bST treatment of the dam increases calf growth. The greatest differences in MR within year for all cows ranged from 24 to 29%. Birth and weaning weights of calves, postpartum BW and BCS of cows, resumption of luteal activity, plasma concentrations of hormones, and ruminal temperature of cows were not influenced by MR. However, concentrations of IGF-I in plasma were negatively correlated with MR at 2 mo post partum. Protein abundance tended to be greater in H for cofilin-2 compared with L cows. Ruminal temperature decreased 1 d before parturition and increased during estrus. Increase efficiency in the cow-calf segment of the beef industry is feasible. These results, the novel description of the proteome of LM in beef cows and the protocol developed to separate the proteins, provide important information for future research. Weaning heavier calves using similar resources, or weaning similar weight calves using less feed, will improve efficiency of beef cattle production and enhance sustainability of the environment.

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